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Studies on YKL-40 in knee joints of patients with rheumatoid arthritis and osteoarthritis. Involvement of YKL-40 in the joint pathology

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Summary

Objective: The presence of YKL-40 (human cartilage glycoprotein 39) in synovium, cartilage and synovial fluid (SF) from knee joints of patients with rheumatoid arthritis and osteoarthritis (OA) were related to histopathological changes in synovium and cartilage and to serum YKL-40 and other biochemical markers.

Methods: The localization of YKL-40 in synovium and cartilage was determined by immunohistochemistry. Synovial inflammation was estimated histologically and by magnetic resonance imaging (MRI). Biochemical markers of inflammation, neutrophil activation and cartilage metabolism were analysed. YKL-40 concentrations in serum and SF were determined by RIA and ELISA.

Results: In the synovium YKL-40 positive cells were found in lining and stromal cells (macrophages) and the number of YKL-40 positive cells was related to the degree of synovitis. In arthritic cartilage, YKL-40 was located to chondrocytes. YKL-40 levels in SF were higher in RA patients with moderate/severe or none/slight synovitis of the knee joint compared to OA patients with moderate/severe or none/slight synovitis. SF YKL-40 correlated with the synovial membrane and the joint effusion volumes determined by magnetic resonance imaging (MRI) and with other biochemical markers of intercellular matrix metabolism. SF YKL-40 was higher than serum YKL-40, and a relationship existed between the YKL-40 levels in SF and serum. Intraarticular glucocorticoid injection was followed by clinical remission and a decrease in serum YKL-40, which increased again at clinical relapse.

Conclusions: YKL-40 in SF is derived from cells in the inflamed synovium, chondrocytes and SF neutrophils. Joint derived YKL-40 influences serum YKL-40. YKL-40 may be involved in the pathophysiology of the arthritic processes and reflect local disease activity. © 2001 OsteoArthritis Research Society International

Key words: YKL-40, Human cartilage glycoprotein 39, Rheumatoid arthritis, Osteoarthritis.

Introduction

YKL-40*, also called human cartilage glycoprotein-39 (HC gp-39)¹, is a mammalian member of family 18 glycosyl hydrolases^{1–8} and has a known gene sequence⁷. YKL-40

is a heparin and chitin-binding lectin^{4,8} without chitinase activity^{1,5,8}. The physiological function of YKL-40 is unknown, but the protein contains several DR4 peptide binding motifs and may be a target of the immune response in rheumatoid arthritis (RA)⁹. Several cells present in the arthritic joint can secrete YKL-40. It is one of the most abundant proteins secreted *in vitro* by chondrocytes^{1,5} and synovial cells² from patients with RA. We have recently reported a high number of YKL-40 positive chondrocytes in articular cartilage from the hip joint of patients with osteoarthritis (OA)¹⁰. YKL-40 positive chondrocytes were in particular located in the superficial and middle layer of the cartilage and especially in areas of the joint with a considerable biomechanical load. Chondrocytes from normal cartilage were mainly YKL-40 negative¹⁰. Another recent study¹¹ demonstrated by *in-situ* hybridization the same zonal distribution of YKL-40 (HC gp-39) in osteoarthritic cartilage, as well as the absence of the protein in normal cartilage. YKL-40 expression by osteophytic tissue in end stage osteoblasts and by primary osteocytes from osteoarthritic patients was also reported¹¹. YKL-40 is not produced by normal monocytes but is secreted by differentiated and activated human macrophages in many different

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*YKL-40 is also named human cartilage glycoprotein-39 (HC gp-39) (Ref. 1), 38-kDa heparin binding glycoprotein (Ref. 4), and CHI3L1 (Ref. 7). The protein had been termed YKL-40 from its molecular weight (40 kDa) and the one letter code for its three NH₂-terminal amino acids (tyrosine, lysine, and leucine) (Ref. 18).

tissues^{6–8,12,13}, including inflamed synovium from patients with active RA⁶. Furthermore, YKL-40 is present in the specific granules of neutrophils and is exocytosed by activation¹⁴.

YKL-40 in serum and synovial fluid can be measured by radioimmunoassay (RIA)³ or enzyme linked immunosorbent assay (ELISA)¹⁵. Increased levels of YKL-40 in serum and synovial fluid are found in patients with active RA^{3,15–16} or severe knee OA¹⁷ compared to normal subjects¹⁷. A correlation exists between the level of YKL-40 in serum and synovial fluid with approximately 10-fold higher values in synovial fluid^{3,17}.

The object of the present study was to elucidate a possible involvement of YKL-40 in the pathophysiology of RA and OA as well as the role of YKL-40 as a marker of disease activity. The presence of YKL-40 in the synovial membrane and articular cartilage was examined by immunohistochemical methods, and the YKL-40 concentrations in synovial fluid and serum were examined by immunoassays (RIA and ELISA) in patients with RA and OA. We related the YKL-40 findings with histopathological changes in the synovial membrane and articular cartilage, with the synovial membrane and the joint effusion volumes [determined by magnetic resonance imaging (MRI)] and with biochemical markers of inflammation, neutrophil activation and cartilage metabolism.

Patients and methods

The investigation included a cross-sectional and a longitudinal study. The knee joint was used as a model and dominating knee joint involvement was an inclusion criteria. The research protocols were approved by the Ethics Committee for Medical Research in Copenhagen. In accordance with the Helsinki Declaration II each patient was informed about the study verbally and in writing, and all gave their written consent.

CROSS SECTIONAL STUDY

Twenty patients with RA and 39 patients with OA were included. The patients were scheduled to undergo total knee joint replacement [RA ($N=13$) and OA ($N=39$)] or arthroscopic synovectomy of the knee joints [RA ($N=7$)]. The patients fulfilled the American College of Rheumatology 1987 classification criteria for RA¹⁹ or the ACR 1986 classification criteria for idiopathic OA of the knee²⁰. Patients with malignant disease or elevated liver enzymes were not included. Seven of the patients with RA were treated with slow-acting antirheumatic drugs (DMARDs) [methotrexate ($N=2$), sulfasalazine ($N=4$), penicillamine ($N=1$)], either as the only treatment ($N=4$), in combination with non-steroidal antiinflammatory drugs (NSAIDs) ($N=1$), or in combination with low dose oral prednisolone ($N=2$). Low dose oral prednisolone (1.25–10.0 mg/day) was given as the only treatment in six patients, and given in combination with NSAID in three patients. Twelve patients were treated with NSAID alone [RA ($N=1$) and OA ($N=11$)]. Three patients with RA did not receive any kind of antirheumatic medicine. Just prior to surgery a clinical examination and blood samples were performed. In case of joint effusion synovial fluid was aspirated by the surgeon when the operation was initiated. Variable amounts of synovial fluid were collected dependent upon the amount available in the joint and the degree of waste in connection with the

collection. Thirty-one of these patients underwent a MRI determination of the knee joint and were included in a project investigating MRI determination of synovial membrane and joint effusion volumes in relation to signs of synovial inflammation²¹.

LONGITUDINAL STUDY

Eighteen patients with RA according to the ACR 1987 classification criteria¹⁹ and clinical signs of knee joint synovitis (15 females and three males) were included. Median disease duration was 5.5 years (range 0.3–36 years) and duration of knee symptoms was 0.6 years (range 0.1–7 years). The patients had a clinical indication for arthrocentesis and intraarticular corticosteroid injection. During arthrocentesis, as much synovial fluid as possible was aspirated, and 80 mg methylprednisolone acetate (2 ml, 40 mg/ml) plus 6 ml lidocaine 0.5% (lidocaine hydrochloride 5 mg/ml) were injected into the knee joint. Serum samples were collected just prior to corticosteroid injection and after 1, 7, 14, 30, 60, 90 and 180 days. Seven patients received continuous therapy with low-dose oral prednisolone (2.5–8.75 mg/day) in combination with DMARD (sulfasalazine ($N=2$) and methotrexate ($N=1$)) and NSAID ($N=3$), or with NSAID alone ($N=2$). Three of the patients were treated with DMARD [penicillamine ($N=2$) and sulfasalazine ($N=1$)] in combination with NSAID, eight patients received NSAID alone. No patients had received intraarticular glucocorticoid therapy within the last 4 weeks.

SYNOVIAL MEMBRANE BIOPSIES

From all patients included in the cross-sectional study, synovial biopsy specimens were obtained during surgery. In patients who had a MRI determination of the synovial membrane and joint effusion volume performed, four pre-selected sites of the synovial membrane of the knee joint²¹ were chosen. Patients, who did not have a MRI determination, had synovial membrane biopsies obtained from three different sites of the knee joint: (1) the lateral; (2) the medial; and (3) the suprapatellar recess. The surgeon selected these three biopsies to represent the overall degree of synovitis of the knee joint [assessed by edema, volume and vascularity (redness) of the synovial membrane]. The synovial biopsy specimens were immediately fixed in 10% neutral buffered formalin, pH 7.0 until the next day, dehydrated in graded mixtures of ethanol and water, immersed in xylene, paraffin embedded, cut at 5 μ m and stained with hematoxylin and eosin.

SYNOVIAL MEMBRANE AND JOINT EFFUSION VOLUMES DETERMINED BY MRI

MRI was performed using a 1.5-Tesla Magnetom (Siemens, Erlangen, Germany) equipped with a dedicated knee coil. Synovial membrane and joint effusion volumes were calculated from continuous pre- and post-gadolinium-DPTA (Shering, Berlin, Germany) T1-weighted transversal MR-images, by means of image processing software. The method is described in detail elsewhere²¹.

HISTOLOGICAL EVALUATION OF SYNOVIAL SPECIMENS

Synovial inflammatory activity was graded under blinded conditions by an experienced histopathologist. The grading

of the inflammatory reaction was done as a general average of all of the synovial tissue from all the biopsies and was based upon a grading of the following nine parameters and described in details elsewhere²¹:

- (1) subsynovial infiltration of polymorphonuclear leukocytes;
- (2) subsynovial infiltration of mononuclear leukocytes;
- (3) surface fibrin deposition;
- (4) multiplication of the synovial lining;
- (5) villous hypertrophy of the synovial surface;
- (6) proliferation of blood vessels;
- (7) perivascular edema;
- (8) formation of granulation tissue; and
- (9) fibrosis.

Each of the nine parameters was graded as 0 (none), 1 (mild), 2 (moderate), or 3 (severe), and an 'average grade' for each parameter was calculated as the average of the grading of the collected biopsies from each knee. Based upon a total score for each knee joint achieved from the average score for all of the nine parameters considered, the knees were divided into two groups: 'None/slight' synovial inflammation ($N=40$) included knees with an average grade ≤ 1 for all signs of inflammatory activity considered; and 'moderate/severe' synovial inflammation ($N=19$) included knees with an average grade >1 for all signs of inflammatory activity considered.

IMMUNOHISTOCHEMICAL STAINING FOR YKL-40 ANTIGEN IN SYNOVIAL MEMBRANE

Alkaline phosphatase staining technique for polyclonal antibodies was used and included the following steps (all performed at room temperature and separated by washes in TBS). Non-specific binding was inhibited by incubation for 20 min with 3% bovine serum albumin (BSA) (Sigma A-4503) in Tris buffered saline (TBS, 5 mM Tris-HCl, 146 mM NaCl, pH 7.4). This buffer was used for dilution of the different antibodies. Incubation for 30 min with an affinity-purified rabbit polyclonal immunoglobulin (IgG) against human YKL-40 (IgG concentration of the YKL-40 antibody was 33 $\mu\text{g}/\text{mL}$). The human YKL-40 used for immunization of rabbits and for affinity purification of antibodies is described elsewhere³. The YKL-40 antibody used in these studies was purified from rabbit antiserum by affinity chromatography using a Sepharose support with covalently attached purified human YKL-40. The antibodies were eluted by 100 mM glycine (pH 2.5). Non-immune rabbit IgG (DAKO X0936, Copenhagen, Denmark) was used as negative control in the same IgG concentration (33 $\mu\text{g}/\text{mL}$). Thereafter incubation for 30 min with alkaline phosphatase-conjugated swine antibodies to rabbit IgG (DAKO D0306, diluted 1:20). Sigma FAST[®] BCIP/NBT tablets (Sigma B-5655; 5-Bromo-4-chloro-3-indolylphosphate/Nitro Blue Tetrazolium) with 0.25 mg/mL Levamisole in 0.1 M Tris-HCl, pH 9.5 was used as color substrate (30 min incubation time). Positive staining was recognized as a dark blue/violet color.

DOUBLE STAINING FOR YKL-40 AND CD68 (MACROPHAGE PROTEIN) IN THE SYNOVIAL MEMBRANE

Peroxidase/DAB (3,3-diaminobenzidine tetrahydrochloride (DAB; KemEnTec, Copenhagen, Denmark) was used as immunostain for the second immunostaining step in the

double staining procedure, which included the following sequences (all performed at room temperature and separated by washes in TBS/Tween20). The sections were pre-incubated with 0.6% H_2O_2 (Perhydrol, Merc, Darmstadt, Germany) in 0.005 M TBS for 30 min, and staining for YKL-40 antigen was performed as described in the section above. After incubation with Sigma FAST[®] BCIP/NBT the color reaction was stopped in distilled water at 4°C. Nonspecific binding was inhibited by incubation for 30 min with 3% BSA in TBS (this buffer was also used for dilution of the various antibodies). Then incubation was performed for 30 min with mouse antihuman CD68 (DAKO M814), diluted 1:50, followed by incubation with a rabbit antimouse IgG (DAKO Z259), diluted 1:20. Non-immune mouse serum (DAKO X0931) was used as a negative control in the same IgG concentration. Then incubation was carried out for 30 min with peroxidase labeled mouse monoclonal PAP [horseradish peroxidase labeled mouse monoclonal antihorseradish peroxidase (DAKO P850)]. Pre-incubation for 10 min with DAB (KemEnTec 4170), 0.5 mg/mL buffer, pH 7.0 was followed by incubation for 10 min in the same DAB solution but with the addition of 0.018% H_2O_2 . The reaction was stopped with distilled water at 4°C for 15 min. Positive staining was recognized as a dark blue/violet color for YKL-40 antigen and a brown color for CD68 antigen.

MICROSCOPIC EVALUATION OF IMMUNOHISTOCHEMICAL STAINING OF SYNOVIAL SPECIMENS

The synovial sections were examined blindly two times by the same observer and scored for the presence of YKL-40 positive cells in a scale as follows: score 0=all cells negative; score 1=few cells positive (approximately 1–25%); score 2=a considerable number of cells positive (approximately 25–75%); score 3=most/all cells positive (approximately 75–100%).

ARTICULAR CARTILAGE BIOPSIES

Standardized samples of cartilage representing all aspects of the joint surfaces (femoral, tibial and patellar surface) of the knee were collected from up to 16 topographically different areas (Fig. 1) from four of the patients with RA (mean age 63 years, range 34–74) and from four of the patients with OA (mean age 77 years, range 74–79) from the cross-sectional study. Only 58 biopsies from the patients with RA and 59 biopsies from the patients with OA were collected, because some areas of the joint surfaces contained only denuded bone. The cartilage samples were stored in cryovials at -80°C until immunohistochemical analysis.

HISTOLOGICAL EVALUATION OF CARTILAGE SPECIMENS

The cartilage sections were evaluated for the presence or absence of histopathological cartilage changes, including surface fibrillation/fissures and clusters/clones²².

IMMUNOHISTOCHEMICAL STAINING FOR YKL-40 ANTIGEN IN CARTILAGE

Four micrometer cryostat cartilage sections were fixed with acetone at room temperature for 15 min. Avidin/

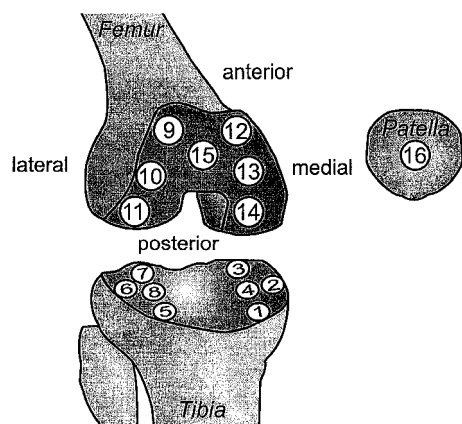


Fig. 1. Standardized samples of cartilage were collected from up to 16 topographically different areas of the arthritic knee joint; *Tibia*: condylus medialis tibiae: 1=anterior, 2=intermediate, 3=posterior, 4=central; condylus lateralis tibiae: 5=anterior, 6=intermediate, 7=posterior, 8=central; *Femur*: condylus lateralis femoris: 9=anterior, 10=intermediate, 11=posterior; condylus medialis femoris: 12=anterior, 13=intermediate, 14=posterior; 15=facies patellaris femoris; *Patella*: 16=facies articularis patellae.

biotinylated horseradish peroxidase staining technique was used as described recently¹⁰. To assess if the accessibility of the YKL-40 epitope of the antibody was hindered by extracellular matrix interactions in the cartilage some of the sections were treated with either protease, 0.05% (type 14, Sigma P5147), trypsin, 0.1% (Sigma T8128) or hyaluronidase, 0.1% (Fluka 531712) for 10 min at 37°C prior to the immunohistochemical staining with an affinity-purified rabbit polyclonal antiserum against human YKL-40 (IgG concentration of 33 µg/mL) followed by incubation with biotinylated swine antirabbit IgG (DAKO E0353, diluted 1:400). Antibody binding was visualized by a complex of avidin-biotinylated horseradish peroxidase (ABComplex/HRP, DAKO K0355) and AEC (3-amino-9-ethylcarbazole) staining kit (SIGMA AEC101). Positive staining was a red color associated with the cytoplasm of the cell.

MICROSCOPIC EVALUATION OF IMMUNOHISTOCHEMICAL STAINING OF CARTILAGE SPECIMENS

Two of the sections [RA ($N=1$) and OA ($N=1$)] were not evaluated due to inadequate quality (tissue folds). The cartilage sections were examined blindly by two observers and scored for the presence of YKL-40 positive chondrocytes in a scale as follows: score 0=all chondrocytes negative; score 1=few chondrocytes positive (1–25% positive); score 2=considerable number of chondrocytes positive (25–75%); and score 3=most/all chondrocytes positive (75–100%).

BIOCHEMICAL ANALYSIS OF SERUM AND SYNOVIAL FLUID

Blood samples were allowed to clot at room temperature and then centrifuged at 2000 *g* for 10 min. Serum was either analysed immediately or stored at –80°C until analysis was performed. The synovial fluid samples were centrifuged at 2000 *g* for 10 min and the cell-free supernatants were stored at –80°C until analysis was performed. ESR in blood was determined by the Westergren method and serum CRP was determined with nephelometry. YKL-40

concentrations in synovial fluid and serum were determined by two different methods; an in-house RIA using polyclonal rabbit antibodies³ and a commercial ELISA using both monoclonal and polyclonal antibodies (Metra Biosystems)¹⁵. The two assays and the normal YKL-40 levels in serum and synovial fluid are described in detail elsewhere^{3,15,17}. The source of the YKL-40 antigen used for standards and production of antibodies in both methods were purified from the conditioned medium of the human osteosarcoma cell line MG63. However, the methods used to calibrate the standards were different. The concentration of the amino-terminal propeptide of type III procollagen was determined by RIA²³. The concentrations in synovial fluid and serum of cartilage oligomeric matrix protein (COMP) and sulfated glucosaminoglycans (GAG) were determined by Wieslab (Lund, Sweden)^{24,25}. The concentrations in synovial fluid of myeloperoxidase²⁶, lactoferrin²⁷, neutrophil gelatinase associated lipocalin (NGAL)²⁸ and human cathelicidin (hCAP-18)²⁹ were determined by ELISA. Due to restricted amounts of serum and synovial fluid available, incomplete number of measurements occurred in some of the variables analysed.

CLINICAL JOINT ASSESSMENT

Clinical assessment of disease activity in the joints included assessment of joint swelling and tenderness. The knee joints as well as the total number of joints involved were registered. Clinical indication for arthrocentesis of the knee joint and subsequent intraarticular corticosteroid injection was determined by clinical signs of intraarticular fluid and of synovitis. Remission was defined by absence of synovial swelling and tenderness of the knee joint. Clinical relapse was defined by recurrence of joint swelling and tenderness.

STATISTICAL ANALYSIS

The statistical analysis was performed with SPSS® (Statistical Package for the Social Science) Software. Results are given as median, range and ratios. Comparison between groups was performed by the non-parametric Mann–Whitney test or the Kruskal–Wallis test (and subsequent Dunn's method). Comparison between two related samples was performed by the Wilcoxon signed ranks test. Subsequent comparison between the consecutive changes over time was performed by regression analysis (random coefficient regression model) using the 'lme' software by Pinheiro and Bates. Correlation analysis between the different parameters was calculated by the Spearman rho test. *P*-values less than 0.05 were considered significant.

Results

The clinical characteristics of the patients are shown in Table I. Patients with RA had more swollen joints, a higher histological score of synovial inflammation and a larger volume of synovial membrane of the knee joint (determined by MRI) than patients with OA. ESR, serum CRP, serum and synovial fluid YKL-40 and PIIINP were highest in patients with RA. Serum YKL-40 levels were higher in RA patients compared to healthy subjects (median 296 µg/L vs 102 µg/L, $P<0.01$) (Table I). No differences between the two groups of patients were found in the synovial fluid concentrations of COMP and GAG. However, serum

Table I
Patient characteristics in the cross-sectional and the longitudinal study of arthritic knee joints

	Cross-sectional study		Longitudinal study	Normal values
	RA	OA	RA	
Age	66 (29–77) ^{20***}	74 (50–85) ³⁹	58 (19–78) ¹⁸	
Number of swollen joints—total	3 (0–18) ^{19***}	0 (0–3) ³⁵	3 (1–30)	
Number of swollen joints—large	2 (0–6) ^{19***}	0 (0–3) ³⁵	2.5 (1–10)	
Number of swollen joints—small	0 (0–13) ^{19***}	0 (0) ³⁵	0 (0–25)	
ESR, mm/h	44 (3–95) ^{20***}	12 (2–92) ³⁵	47 (2–105)	<20
Serum CRP, nmol/L	246 (30–1413) ^{20***}	89 (30–419) ³⁷	327 (29–777)	<90
Serum YKL-40 (RIA), µg/L	296 (55–920) ^{19*}	130 (56–1290) ³¹	276 (104–540)	<208 (Ref. 17)
Serum YKL-40 (ELISA), µg/L	87 (20–218) ¹²	73 (26–565) ³¹		<95 (Ref. 15)
Serum COMP, mg/L	0.9 (0.6–1.3) ^{14**}	1.2 (0.7–1.7) ²²		
Serum PIIINP, µg/L	4.5 (1.5–8.4) ^{19**}	3.4 (1.4–6.0) ³¹		<3.5
Synovial inflammation, 0–1	1 (0–1) ^{20***}	0 (0–1) ³⁹		
MRI/synovial membrane volume, cm ³	69 (21–93) ^{10*}	36 (8–79) ²¹		
MRI/joint effusion volume, cm ³	40 (4–232) ¹⁰	21 (0–119) ²¹		
SF YKL-40 (RIA), µg/L	4890(910–13800) ^{20***}	2700 (351–6000) ³⁹		<1350 (Ref. 17)
SF YKL-40 (ELISA), µg/L	2120 (485–6850) ^{14**}	1190 (274–2600) ²⁹		<402 (Ref. 15)
SF PIIINP, µg/L	3230 (600–6700) ^{19***}	960 (240–3310) ³⁷		
SF COMP, mg/L	7.2 (3.1–22.1) ¹⁸	9.2 (3.9–20.5) ³⁴		
SF GAG, mg/L	42 (10–132) ¹⁶	42 (3–79) ³³		
Ratio SF/serum YKL-40 (RIA)	20 (2–67) ¹⁹	13 (3–55) ³¹		
Ratio SF/serum YKL-40 (ELISA)	16 (8–112) ³⁹	15 (3–56) ³³		
Ratio SF YKL-40 (RIA)/SF COMP	548 (122–2721) ^{17**}	247 (38–939) ³⁴		
Ratio SF YKL-40 (RIA)/SF GAG	156 (15–540) ^{16*}	63 (8–530) ³³		
Ratio SF YKL-40 (ELISA)/SF COMP	295 (34–991) ^{14**}	144 (19.7–440) ²⁹		
Ratio SF YKL-40 (ELISA)/SF GAG	75 (11–234) ¹³	34 (10–200) ²⁸		

Values are given as median and ranges^{number of patients}. CRP=C-reactive protein; ESR=erythrocyte sedimentation rate; PIIINP=amino terminale propeptide of type III procollagen; COMP=cartilage oligomeric matrix protein; GAG=glycosaminoglycans; MRI=magnetic resonance imaging. SF=synovial fluid; synovial inflammation (0=none/slight, 1=moderate/severe). *= $P<0.05$; **= $P<0.01$; ***= $P<0.001$ vs patients with OA tested by the Mann–Whitney U-test.

COMP was significant higher in OA patients compared to RA patients ($P<0.01$) (Table I).

SYNOVIAL FLUID YKL-40 LEVELS IN RELATION TO HISTOLOGICAL ASSESSMENT OF SYNOVIAL INFLAMMATION

Highest levels of YKL-40 in synovial fluid were found in knee joints from patients with RA and moderate to severe degree of synovial inflammation (median 6300 µg/L) and the levels were higher ($P=0.054$) compared to patients with OA and moderate to severe synovial inflammation (3400 µg/L) (Table II and Fig. 2). Patients with RA and none to slight synovial inflammation had higher levels of YKL-40 in synovial fluid compared to OA patients with none to slight synovitis (median 4200 µg/L vs 2450 µg/L, $P=0.026$). Similar results were found when the ELISA method was used to measure YKL-40. There was a trend towards higher SF YKL-40 levels in RA patients with moderate to severe synovial inflammation compared to those with none to slight synovitis; however, the difference was not significant ($P=0.096$).

IMMUNOHISTOCHEMICAL STAINING OF YKL-40 IN SYNOVIAL MEMBRANE IN RELATION TO SYNOVIAL INFLAMMATION AND SYNOVIAL FLUID YKL-40 LEVELS

Positive staining for CD68 antigen was observed in cells of the synovial lining and in mononuclear cells in the stroma of the synovial membrane in patients with RA and OA, and some of these cells were also positive for YKL-40 antigen

[Fig. 3(A)]. The number of YKL-40 positive cells was related with the degree of synovial inflammation. A severely inflamed synovial membrane had a higher number of YKL-40 positive cells compared to a less inflamed synovial membrane. No difference was observed in the staining pattern of synovial membranes of RA and OA patients. The score of YKL-40 positive cells in the synovium gained from 0 (no positive cells) to 2 (25–75% positive cells) (Table III). No sections had positive YKL-40 staining in all cells. YKL-40 positive cells were found in 29% of the sections with none to slight synovial inflammation and in 70% of the sections with moderate to severe synovial inflammation. The level of YKL-40 in synovial fluid was related to the score of YKL-40 positive cells in the synovial membrane (Fig. 4) (Kruskal–Wallis test, $P=0.0156$, $N=22$) with a significant difference (Dunn's method, $P<0.05$) between synovial membranes with the higher (score=2) and the lower (score=0) number of YKL-40 positive cells.

SYNOVIAL FLUID YKL-40 LEVELS IN RELATION TO THE SYNOVIAL MEMBRANE AND JOINT EFFUSION VOLUMES DETERMINED BY MRI

The concentration of YKL-40 in synovial fluid was significantly correlated with the MRI-determined volumes of the synovial membrane ($\rho=0.64$, $P<0.001$, $N=31$) (Fig. 5) as well as the joint effusion volumes ($\rho=0.59$; $P<0.001$, $N=31$) in patients with RA and OA. A significant correlation was found between serum PIIINP and the volume of the synovial membrane ($\rho=0.39$, $P<0.05$) and of the joint effusion determined by MRI ($\rho=0.33$; $P=0.07$). Synovial fluid levels of COMP, GAG and the markers of different

Table II
The synovial fluid concentration of the biochemical markers in relation to the degree of synovial inflammation of the knee joint in patients with RA and OA

	RA		OA	
	None/slight	Moderate/severe	None/slight	Moderate/severe
Synovial inflammation				
Number of patients	7	13	33	6
YKL-40 (RIA), µg/L	4200 (1450–5900) ⁷	6300 (910–13800) ¹³	2450 (351–4700) ³³	3400 (1400–6000) ⁶
YKL-40 (ELISA), µg/L	1865 (485–2360) ⁶	3340 (1540–6850) ⁸	1180 (274–2470) ²³	1795 (835–2600) ⁶
PIIINP, µg/L	3230 (650–6540) ⁷	3085 (600–6700) ¹²	940 (240–3310) ³¹	1135 (670–2530) ⁶
COMP, mg/L	13.6 (5.1–22.1) ⁷	6.8 (3.1–20.5) ^{11*}	9.3 (4.5–20.5) ²⁸	8.1 (3.9–9.5) ⁶
GAG, mg/L	65 (10–132) ⁷	30 (18–49) ^{9*}	58 (3–79) ²⁷	22 (13–60) ⁶
Ratio YKL-40/COMP	222 (123–1059) ⁷	1186 (193–2721) ^{10**}	230 (38–640) ²⁸	551 (148–939) ⁶
Ratio YKL-40/GAG	55 (15–540) ⁷	228 (97–387) ^{9*}	56 (8–530) ²⁷	115 (48–462) ^{6*}
Ratio YKL-40 (ELISA)/COMP	110 (34–459) ⁶	518 (123–991) ^{8*}	125 (20–440) ²³	259 (88–419) ^{6*}
Ratio YKL-40 (ELISA)/GAG	20 (11–234) ⁶	91 (62–228) ^{7*}	30 (10–163) ²²	84 (29–200) ^{6*}
NGAL, µg/L	66 (19–116) ³	377 (12–591) ⁵	30 (17–47) ¹⁸	61 (29–92) ²
Lactoferrin, µg/L	831(30–10110) ⁶	771 (228–1345) ⁴	112 (30–7106) ²⁵	596 (94–6232) ⁴
h-CAP 18, µg/L	1678 (127–7305) ⁵	2034 (461–3453) ⁴	128 (49–1619) ³¹	494 (115–879) ⁴
MPO, µg/L	33 (14–208) ⁴	219 (51–461) ⁶	60 (16–647) ²⁶	59 (52–410) ³

Values are given as medians and ranges^{number of patients}. RIA=radioimmunoassay; ELISA=enzyme linked immunosorbent assay; PIIINP=amino terminal propeptide of type III procollagen; COMP=cartilage oligomeric matrix protein; GAG=glycosaminoglycans; markers of neutrophil activation: NGAL=neutrophil gelatinase associated lipocalin; lactoferrin; cathelicidin h-CAP 18; MPO=myeloperoxidase. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$ vs none/slight synovial inflammation tested by the Mann-Whitney U-test.

granules in neutrophils did not correlate statistically with the volume of the synovial membrane and joint effusion.

IMMUNOHISTOCHEMICAL STAINING OF YKL-40 IN ARTICULAR CARTILAGE IN RELATION TO HISTOPATHOLOGICAL CARTILAGE CHANGES

All the arthritic cartilage sections had considerable histopathological changes, including surface fibrillation or fissures and/or clones or clusters. In the pannus-invaded cartilage [Fig. 3(B)] a prominent YKL-40 staining was shown in the cytoplasm of the different cells types present in the pannus, and the extracellular matrix also exhibited some staining [Fig. 3(C)]. We did not attempt to differentiate between the YKL-40 staining in the different cell types. The extracellular matrix in the underlying cartilage was YKL-40 negative around both YKL-40 positive and negative chondrocytes [Fig. 3(C)]. The cartilage from patients with RA exhibited frequently prominent fibrous pannus and clusters/clones [Fig. 3(C)] compared with cartilage from OA patients [Fig. 3(D)]. YKL-40 was found in the cytoplasm of the chondrocytes [Fig. 3(E)] but not in the extracellular matrix, except in areas of pannus-invaded cartilage. Treatment with protease, trypsin or hyaluronidase prior to the immunohistochemical staining did not reveal any overt additional YKL-40 staining of the extracellular cartilage matrix or in the chondrocytes. YKL-40 positive chondrocytes were observed in all layers of the cartilage. Most of the sections included non-intact residual cartilage with only the middle and/or the deep layer left, and no differences in the staining pattern between the different layers could be identified. Chondrocytes located in clusters/clones and in areas of excessive surface fibrillation showed a similar pattern of YKL-40 staining as found in isolated chondrocytes and chondrocytes in other areas of the same section. No difference was found between the YKL-40 staining pattern in articular cartilage in RA and OA patients. Most cartilage sections had a score of 1 (1–25% positive chondrocytes). Seven sections had a YKL-40 score of 2

(25–75% positive chondrocytes) (three sections from RA patients and four sections from OA patients), and no sections had a score of 3. Arthritic knees without YKL-40 staining in chondrocytes were not found. There was no difference in respect of topographic site in the knee joint and the YKL-40 staining (Table IV).

IMMUNOHISTOCHEMICAL STAINING OF YKL-40 IN ARTICULAR CARTILAGE IN RELATION TO SYNOVIAL INFLAMMATION AND SYNOVIAL FLUID YKL-40 LEVELS

YKL-40 staining in chondrocytes from knees with moderate to severe synovial inflammation was similar to the YKL-40 staining in chondrocytes from knees with none to slight synovial inflammation. Two of the knees from which cartilage specimens were obtained were graded with moderate to severe synovial inflammation (both knees were from RA patients) and these knees had high SF YKL-40 levels (7830 and 13 800 µg/L). Two knees were graded with no signs of synovial inflammation (both from OA patients) but the YKL-40 concentration in the synovial fluid was higher (1991 and 4200 µg/L) than the level in normal knees (<1350 µg/L).¹⁷

SYNOVIAL FLUID YKL-40 LEVELS IN RELATION TO BIOCHEMICAL MARKERS OF SYNOVIAL INFLAMMATION, CARTILAGE DEGRADATION AND ACTIVATED NEUTROPHILS

In patients with RA or OA, no significant correlation existed between SF levels of YKL-40 as compared with PIIINP, COMP, GAG or the markers of the specific granules in neutrophils. The concentrations of PIIINP, COMP, GAG, lactoferrin, NGAL and hCAP-18 (located to the specific granules as YKL-40) were not different in the synovial fluid from patients with moderate to severe synovial inflammation and patients with none to slight synovial inflammation (Table II), neither in patients with RA nor OA.

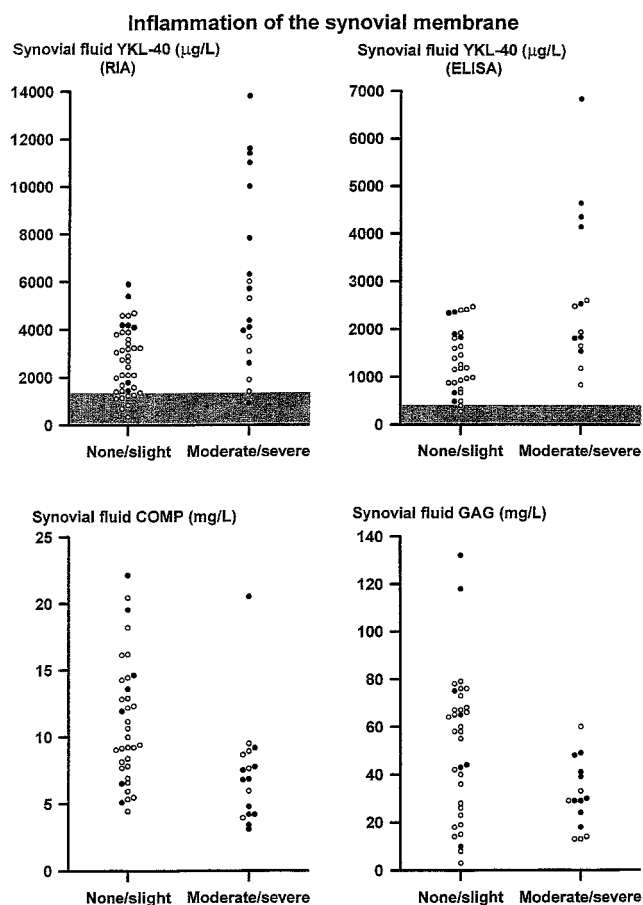


Fig. 2. Synovial fluid concentrations of YKL-40 (determined by radioimmunoassay (RIA) and ELISA, respectively), COMP, and GAG in relation to the degree of synovial inflammation determined by histological criteria. ●=patients with RA and ○=patients with OA. COMP=cartilage oligomeric matrix protein; GAG=glycosaminoglycans. The hatched areas represent the normal values of the different parameters. Normal values of COMP and GAG were not available.

To minimize the influence of variable amounts of synovial fluid present in the knee joints we also calculated the synovial fluid results as ratios. The median values of the ratios of YKL-40/COMP and YKL-40/GAG were significantly higher in RA knees with moderate to severe synovial inflammation (Table II) compared with RA knees with none to slight synovial inflammation ($P=0.024$ and $P=0.025$, respectively), whereas significant differences between the groups in patients with OA were only found for the ratio of YKL-40/GAG ($P=0.045$) and not for the ratio YKL-40/COMP.

RELATIONSHIP BETWEEN YKL-40 LEVELS IN SYNOVIAL FLUID AND SERUM

YKL-40 in synovial fluid correlated significantly with serum YKL-40 ($\rho=0.46$, $P=0.05$, $N=19$) in patients with RA as well as in patients with OA ($\rho=0.50$, $P=0.004$, $N=31$). The median ratio of YKL-40 in synovial fluid and serum was 19.8 in patients with RA and 12.8 in patients with OA (Table I). Furthermore, significant correlations were found between SF YKL-40 and the ESR ($\rho=0.68$, $P=0.008$) and serum CRP ($\rho=0.62$, $P=0.003$) in patients

with RA, but not in patients with OA. Serum YKL-40 correlated with serum CRP ($\rho=0.56$, $P=0.02$) in patients with RA. The serum and synovial fluid levels of YKL-40 were highest using the in-house RIA. Highly significant correlations were found between both the serum values and the synovial fluid levels of YKL-40 when the results of the two methods were compared (synovial fluid: $\rho=0.77$, $P<0.001$ and serum: $\rho=0.90$, $P<0.001$).

CHANGES IN SERUM YKL-40 AFTER INTRAARTICULAR CORTICOSTEROID

Serum YKL-40 was initially 276 µg/L and significantly higher in the RA patients ($P<0.001$) compared with the value of healthy controls¹⁷. Following intraarticular corticosteroid injection clinical remission occurred. Serum YKL-40 decreased significantly after only 1 day (median 180 µg/L, $P=0.03$). After 7 and 14 days serum YKL-40 was decreased by 15.7% ($P=0.017$) and 18.4% ($P=0.025$) (Table V). However, when a separate test (random coefficient regression model) for an initial effect of glucocorticoid injection and for a trend in the subsequent development (taking into account a possible variation of these effects between persons), the initial effect of glucocorticoid was found to be significant ($P=0.0018$), but the subsequent trend with sustained decrease at day 7 and 14 with return to baseline at day 30 was non-significant ($P=0.127$). Eight patients had a clinical relapse during the next 6 months and these patients had an increase in serum YKL-40 at the time of relapse (350 µg/L) compared with the previous value (224 µg/L, $P<0.05$). Serum PIIINP was also decreased 1 day after glucocorticoid injection (Table V). After 7 days the serum PIIINP concentrations were also significantly decreased, but after 14 days the values were not different from the baseline values. Significant decreases in serum CRP were seen at day 1, 7, and 14 and in ESR at day 7 and 14 after glucocorticoid injection (Table V).

Discussion

The present study demonstrated high concentrations of YKL-40 in synovial fluid of the knee joint of patients with RA and OA. By immunohistochemical methods we demonstrated the presence of YKL-40 in synovial cells of the inflamed synovial membrane with a positive relation between the number of YKL-40 positive cells and the severity of the synovial inflammation. In addition, the MRI-determined volume of the synovial membrane and joint effusion, an estimation of the degree of synovial inflammation, was also related to the levels of YKL-40 in synovial fluid. Others have reported that activated macrophages secrete YKL-40 *in vitro*^{6-8,12,13} and *in-situ* hybridization studies have demonstrated YKL-40 expression in macrophages located in the synovial membrane of patients with RA⁶. Our demonstration of macrophage CD68 positive cells in the inflamed synovial membrane, also positive for YKL-40 antigen, is consistent with the macrophage as a source of secretion of YKL-40 into the synovial fluid. Furthermore, in RA patients a positive relation between the degree of synovial inflammation and the ratios of YKL-40/COMP in synovial fluid was shown, with highest synovial fluid levels of YKL-40 in knees with the highest degree of synovial inflammation. The findings provide evidence that YKL-40 is released from the synovial membrane under

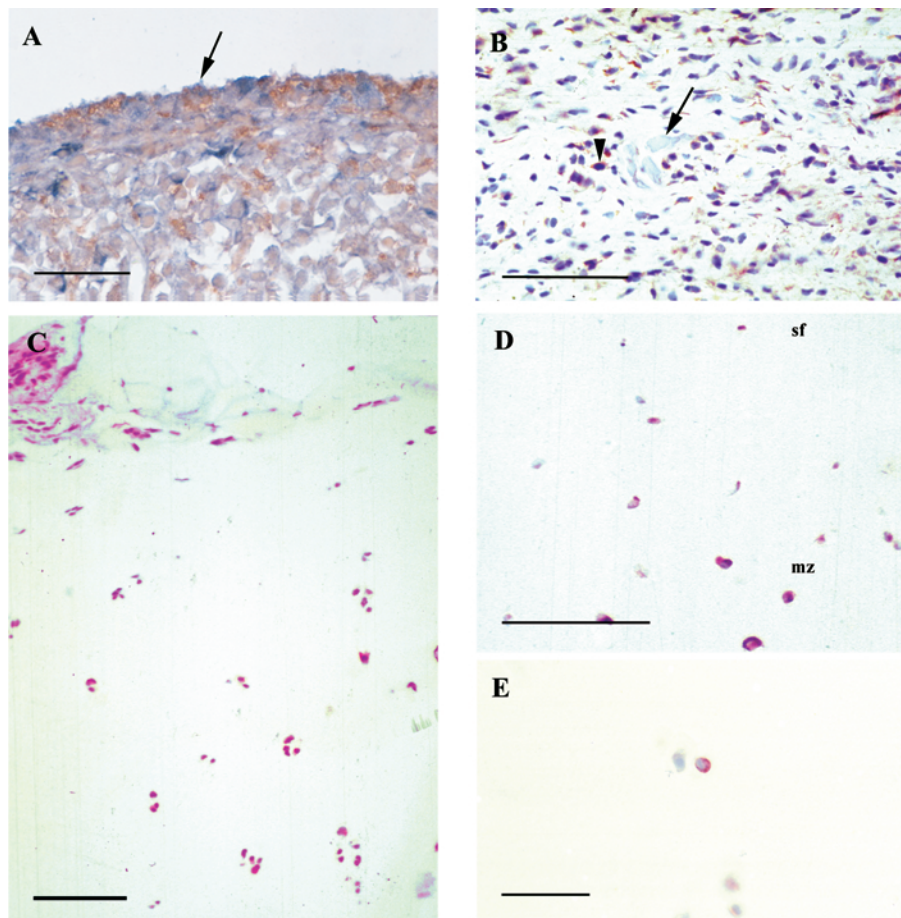


Fig. 3. Representative light micrographs of immunohistochemical staining of YKL-40 in synovial membranes and cartilage from knee joints of patients with RA and OA. (a) A moderately to severely inflamed synovial membrane from a patient with OA. Some of the macrophage CD68 positive cells (brown/red color) were also positive for YKL-40 antigen (dark blue/violet color) as illustrated in the double stained section from a patient with OA and moderate to severe synovial inflammation. The arrow indicates a double stained cell (bar=40 μ m). (b) A pannus from a patient with RA illustrating YKL-40 positive staining of the cells. The arrowhead indicates an YKL-40 positive mononuclear cell. The arrow indicates disintegrated cartilage (bar=100 μ m). (c) Pannus-covered cartilage section rich in clones (arrow) from location no. 10 (see Fig. 1) of the knee joint of a patient with RA, illustrating YKL-40 staining in the cytoplasm of the chondrocytes. No staining is detectable in the cartilage matrix (bar=100 μ m). However, the pannus as well as the pannus-invaded cartilage shows diffuse YKL-40 staining in the extracellular matrix. (d) Osteoarthritic cartilage section from location no. 7 (see Fig. 1), illustrating YKL-40 positive chondrocytes through the superficial zone (sf) and middle zone (mz) of the cartilage. YKL-40 positive chondrocytes are also found in the deep zone of the cartilage (not shown) (bar=100 μ m). (e) A high magnification of a chondrocyte from (D) showing the granular distribution of YKL-40 in the cytoplasm of the chondrocyte (bar=40 μ m).

pathophysiological conditions. However, the high amounts of YKL-40 detectable in the synovial fluid may also originate from other sources, including chondrocytes and activated neutrophils.

In the articular cartilage from the arthritic knee joints YKL-40 was discovered in the cytoplasm of the chondrocytes, in accordance with two recent studies on cartilage from osteoarthritic hip joints^{10,11}. It is known that chondrocytes secrete YKL-40 *in vitro*^{1,5}, and one would expect to find YKL-40 in the articular extracellular matrix. However, YKL-40 staining in the extracellular matrix of cartilage was only found in areas of pannus-invaded cartilage. Enzyme digestion of the cartilage sections prior to the immunohistochemical staining did not expose YKL-40 staining in the extracellular matrix. The presence of YKL-40 in the pericellular matrix or extracellular matrix can not be excluded, since YKL-40 may be bound in a way that prevents its detection or the protein may be present in too low a concentration to be detected by our antibody. Unfortu-

nately, we did not have the opportunity to obtain 'normal' cartilage from non-arthritic knee joints. In a recent study of arthritic and normal cartilage of hip joints¹⁰ we showed that chondrocytes from normal cartilage were, in general, YKL-40 negative. The finding is in accordance with the studies of Hakala *et al.*¹ and Connor *et al.*¹¹, demonstrating no YKL-40 mRNA expression by normal human chondrocytes. A few of the studied arthritic knees showed no signs of synovial inflammation; nevertheless, the synovial fluid YKL-40 level was higher compared to normal knee joints. The observations may indicate that chondrocytes of the arthritic cartilage contributes to the high level of YKL-40 in synovial fluid of the knee joints from patients with RA and OA. The findings are in accordance with our previous reported hypothesis that YKL-40 may play a role in cartilage remodeling in arthritic joints¹⁰.

COMP, a non-collagenous glycoprotein belonging to the heterogeneous family of thrombospondin³⁰ was originally isolated from cartilage. COMP levels in synovial fluid and

Table III
Number of YKL-40 positive cells in the synovial membrane (evaluated as a score) from knee joints of patients with rheumatoid arthritis and osteoarthritis

Score	0	1	2	3
Sections from patients with RA (N=10)	20%	20%	60%	0%
Sections from patients with OA (N=14)	79%	14%	7%	0%
Sections from patients with none/slight synovitis (N=9)	71%	21%	7%	0%
Sections from patients with moderate/severe synovitis (N=15)	30%	10%	60%	0%

Values are given as percentage of the number of sections with a given score in relation to the total of number of sections evaluated. Score 0=all cells negative (0%); score 1=few cells positive (1–25%); score 2=a considerable number of cells positive (25–75%); score 3=most/all cells positive (75–100%).

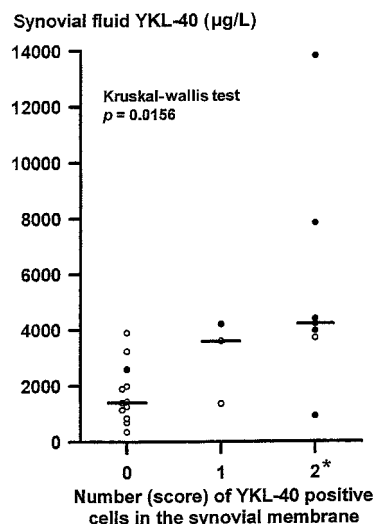


Fig. 4. YKL-40 concentrations in synovial fluid in knee joints of patients with RA (●) and OA (○) in relation to the number of YKL-40 positive cells in the synovial membrane. 0=all cells negative; 1=few cells positive (1–25% positive cells); 2=a considerable number of positive cells (25–75% positive cells). A significant relation was found between the number of YKL-40 positive cells in the synovial membrane and the YKL-40 concentration in the synovial fluid (Kruskal–Wallis test: $P=0.0156$). The significant difference was revealed between SF YKL-40 and synovial membranes with a score=2 and a score=0 (Dunn's method, $P<0.05$). The horizontal bars show the median values of each group.

serum may be related to cartilage damage^{31–33}. However, COMP cannot be regarded as a cartilage-specific protein, since it is also found in tendon, meniscus, cruciate ligaments^{34–36} and is secreted *in vitro* by synovial fibroblasts³⁵. *In-situ* hybridization has shown expression of COMP mRNA in the synovial sublining cells of inflamed synovium from patients with RA.³⁵ In contrast to YKL-40, the COMP expression was not detected in CD68 positive cells of the synovial membrane, but restricted to fibroblasts.³⁵ In the present study serum, but not SF COMP levels were significantly higher in patients with OA compared with patients with RA. A quantitative analysis of the amount of articular cartilage was not performed in our study. However, the inverse relation between the synovial fluid levels of YKL-40 and COMP and GAG may indicate that the release of YKL-40 from the cartilage is inferior to the release of YKL-40 from the synovium. We found a significant difference between the SF ratios YKL-40/COMP in RA patients with moderate to severe synovial inflammation compared to RA patients with none to slight synovial inflammation. A trend towards higher concentrations of

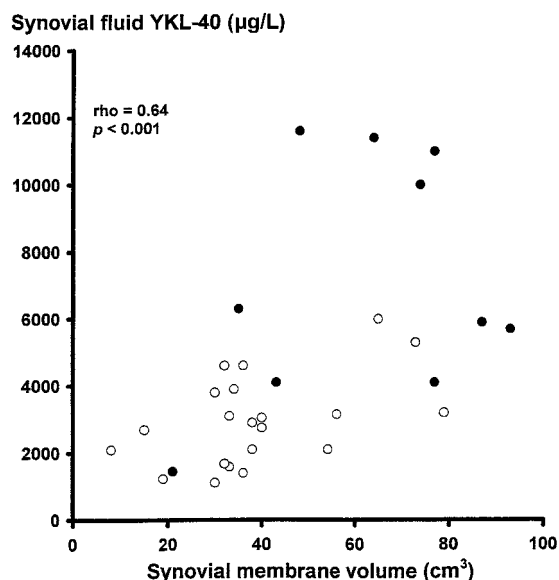


Fig. 5. Synovial fluid concentrations of YKL-40 in relation to the volume of the synovial membrane determined by magnetic resonance imaging.

YKL-40 in synovial fluid in patients with RA and moderate to severe synovial inflammation compared with none to slight inflammation was found. However, the difference was not significant. The significant difference in the ratio YKL-40/COMP between the two groups of synovial inflammation in RA patients may be due to the YKL-40 levels corrected for the influence of variable amounts of synovial fluid present in the different joints by estimating the values of connective tissue markers as ratios.

Neutrophils are important in the pathogenesis of RA^{37,38}. These cells comprise more than 90% of the cells in the synovial space of joints from patients with active RA and have a turnover rate of more than a billion cells per day in an inflamed knee joint³⁹. The neutrophils are also present at the cartilage–pannus junction and can release proteases and biologically destructive oxidative products that can damage cartilage and fibrous tissue, resulting in joint damage^{39–42}. YKL-40 is present in the specific granules of neutrophils and is exocytosed *in vitro* upon activation¹⁴. In the present study we found that hCAP-18, another protein exocytosed from the specific granules of neutrophils, showed a trend towards highest levels in the patients with moderate to severe synovial inflammation of the knee joint, but there were only a few patients in each group ($N=4$ and $N=5$) and no statistical difference was achieved. These observations and the fact that the different organelles of the

Table IV
Topographical distribution of YKL-40 positive chondrocytes in cartilage from arthritic knee joints (RA and OA)

Location no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Median score	1 ⁶	0.5 ⁸	0 ⁷	0.5 ⁸	0 ⁸	1 ⁷	0.5 ⁸	1 ⁷	1 ⁷	1 ⁸	1 ⁷	1 ⁷	1 ⁷	0 ⁸	0.5 ⁸	0.5 ⁶
Range	0–2	0–1	0–1	0–1	0–1	0–1	0–2	0–2	0–2	0–2	0–1	0–2	0–1	0–1	0–2	0–1

Values are given as median scores^{number of sections evaluated} and ranges. The location numbers are presenting the topographical locations of the knee joint for the cartilage samples (see Fig. 1).

Table V
The changes in four different serological variables of synovial inflammation following intra-articular glucocorticoid injection (GCC) in knee joints of patients with RA

	Time of intraarticular GCC	Day 1	Day 7	Day 14	Day 30
Serum YKL-40 (µg/L)	276 (104–480) ¹⁸	180 (108–485) ^{17*}	164 (84–414) ^{15*}	172 (54–470) ^{15**}	179 (56–500) ¹⁴
Serum PIIINP (µg/L)	4.2 (2.2–8.6) ¹⁴	2.9 (1.1–5.1) ^{13***}	2.9 (1.7–5.0) ^{13**}	3.7 (2.1–8.3) ¹¹	3.6 (2.3–11.9) ¹⁰
Serum CRP (nmol/L)	329 (29–1016) ¹⁷	210 (29–1547) ^{15*}	64 (29–327) ^{15***}	166 (29–520) ^{14***}	126 (29–1616) ¹⁵
ESR (mm/h)	46 (2–105) ¹⁷	46 (2–130) ¹⁵	30 (2–84) ^{13**}	28 (8–100) ^{14*}	41 (4–109) ¹⁵

Values are given as medians and ranges^{number of patients}. The data shown are only from patients who did not suffer from relapse of knee joint synovitis in the 30 days time period. *= $P<0.05$; **= $P<0.01$; and ***= $P<0.001$ vs value at time of intraarticular glucocorticoid injection by Wilcoxon signed rank test.

neutrophils are exocytosed in a strict hierarchy⁴¹, suggest that some of the YKL-40 present in the synovial fluid from patients with moderate to severe synovitis originates from neutrophils in the joint. However, the ratios between YKL-40 and the other granule proteins of neutrophils in the synovial fluid were all higher as compared with the ratios that were calculated by results from a recent study where the granule proteins were mobilized from neutrophils in suspension in response to stimulation¹⁴. This finding indicates that synovial fluid YKL-40 also originates from sources other than neutrophils.

In the present study we used two different methods^{3,15} to determine the YKL-40 levels in synovial fluid and serum. We do not know if the two antibodies used in the ELISA method recognize the same epitope on the YKL-40 antigen as recognized by the polyclonal antibody used in our in-house YKL-40 RIA. We found a good correlation between YKL-40 determined by the two methods. There was, however, a difference between the exact levels of YKL-40. This may be explained by differences in the calculation of the standard used in the two assays.

RA is a polyarticular disease. Correlations between concentrations of components in synovial fluid and serum therefore reflect changes in the global amount of synovial fluid and synovial tissue. The positive correlations between serum YKL-40 and YKL-40 in the synovial fluid of the knee joints accords with the dominating knee joint synovitis in the present study and the quantitative dominance of synovial tissue in the knee joint. The ratios of YKL-40 in synovial fluid to serum did not differ between patients with RA and OA reflecting the non-specific nature of joint inflammation. The synovial fluid concentrations of YKL-40 are determined by the release of YKL-40 to the synovial fluid, as well as by the clearance of YKL-40 from the joint cavity, which is unknown so far.

Intraarticular glucocorticoid injection was followed by a decrease in clinical signs of synovitis and a decrease of serum YKL-40. Similarly, a clinical relapse of the knee synovitis was accompanied by an increase in serum YKL-40. The findings demonstrate that local synovitis-

derived YKL-40 influences serum YKL-40. Similar results were found for serum PIIINP though the values returned to baseline earlier than the values of YKL-40 did. The mechanism by which glucocorticoid influences YKL-40 production is unknown. The changes in serum YKL-40 were positively correlated with changes in the serum levels of PIIINP, CRP and ESR, though following a somewhat different course. When the synovial membrane is inflamed, the synthesis as well as degradation rate of type III collagen is increased⁴³. PIIINP is a marker of type III collagen synthesis and is synthesized and secreted by synovial cells⁴⁴. We found high concentrations of SF PIIINP in patients with RA, which probably reflect an increased turnover of type III collagen in the inflamed synovium. The positive correlation between YKL-40 and PIIINP supports the assumption that YKL-40 in part originates from the inflamed synovial membrane. CRP and ESR, the gold standards of biochemical assessment of the disease activity in RA, are produced in the liver by the hepatocytes and are thus an indirect measure of joint inflammation. By contrast, serum YKL-40 is a more direct measure of joint inflammation. Future studies may reveal the potential of serum YKL-40 to provide new information on disease activity and pathophysiology of the synovitis in RA.

In conclusion, our study showed that YKL-40 was detected in the inflamed synovial membrane and the number of YKL-40 positive cells was related with the degree of synovial inflammation. Apart from YKL-40 derived from the synovial membrane, YKL-40 derived from articular cartilage and neutrophils in the synovial fluid may contribute to SF YKL-40. A relationship exists between YKL-40 in serum and synovial fluid with approximately 15-fold higher levels in synovial fluid. Intraarticular glucocorticoid injection, inducing remission in joint inflammation, was followed by a decrease in serum YKL-40. The findings are consistent with a local release of YKL-40 in the arthritic joint influencing serum YKL-40. Assessment of serum YKL-40 may provide new and direct information on the local disease activity as well as on the pathophysiological processes in the arthritic joint.

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