Serum YKL-40 concentrations in patients with rheumatoid arthritis: relation to disease activity

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Abstract

Objective. YKL-40, also called human cartilage glycoprotein-39, is secreted by chondrocytes, synovial cells, macrophages and neutrophils. Studies have shown that YKL-40 is an autoantigen in rheumatoid arthritis (RA). We evaluated whether serum YKL-40 was related to disease activity in patients with RA.

Methods. Serum YKL-40 was determined by radioimmunoassay in 156 patients with RA during a 1 yr longitudinal study.

Results. Serum YKL-40 was increased in 54% of the patients with clinically active disease. Patients with clinically active disease initially who became inactive after 12 months had a significant decrease in serum YKL-40 (-30%, $P < 0.002$) and patients who changed from inactive to active disease had an increase in serum YKL-40. Patients who remained active had unchanged serum YKL-40 during the study. Serum YKL-40 decreased rapidly (-24% after 7 days, $P < 0.01$) during prednisolone therapy, and more slowly in patients treated with methotrexate only (-15% after 60 days, $P < 0.01$). Patients with early RA (disease duration < 3 yr, $n = 50$) and a persistently elevated serum YKL-40 were at risk of radiological disease progression as determined by Larsen score.

Conclusion. Serum YKL-40 varies according to disease activity in RA, but provides in some respect information different from conventional markers. Our previous studies are consistent with a local release of YKL-40 in the arthritic joint followed by a secondary increase in serum YKL-40. YKL-40 may prove to be a new tool for the study of disease activity and pathophysiology of RA.

KEY WORDS: YKL-40, Rheumatoid arthritis, Disease activity, Human cartilage glycoprotein-39.

The aetiology of rheumatoid arthritis (RA) is unknown. However, autoimmune mechanisms play a central role in the pathogenesis of the disease [1]. A prevailing view is that the chronic inflammation in patients with RA is mediated by antigen-induced activation of T cells infiltrating the synovial membrane, which then induces inflammatory processes resulting in synovial proliferation associated with resorption of cartilage and bone [2–4].

YKL-40 (the protein has been termed YKL-40 from its molecular weight (40 kDa) and the one-letter code for its three N-terminal amino acids [16]), also named human cartilage glycoprotein-39 (HC gp-39) [5], is a mammalian member of the family of 18 glycosyl hydrolases which includes bacterial chitinases [5–10].

Verheijden *et al.* [11] have recently reported that HC gp-39 contains several DR4 peptide binding motifs and that it may be a target of the immune response in RA. When HC gp-39 was injected into BALB/c mice, a chronic relapsing arthritis was found and inhalation of the protein led to tolerization of antigen-specific T cells and in suppression of the HC gp-39 induced arthritis. The physiological function of YKL-40 is unknown. YKL-40 is a chitin-binding lectin [12], but has no chitinase activity. The pattern of its expression in normal and disease states suggests that YKL-40 may play a role in remodelling or degradation of the extracellular matrix and in the inflammatory process [5–20]. YKL-40 mRNA expression is found in human articular cartilage and synovium from patients with RA, but cannot be detected in articular cartilage from normal subjects [5, 7]. In primary cultures of articular cartilage chondrocytes and synovial cells from patients with RA or osteoarthritis, YKL-40 is one of the most abundant proteins found in conditioned culture medium [5, 7, 13].

In situ hybridization studies of inflamed synovial tissue from patients with RA have demonstrated that YKL-40 is expressed in macrophages *in vivo* [14]. YKL-40 is expressed *in vitro* by activated macrophages [8, 12, 14] and is exocytosed by activation from the specific granules of neutrophils [15].

In a previous study, we have reported increased serum concentrations of YKL-40 in patients with RA, a 10-fold increase in the synovial fluid concentration compared to serum YKL-40, and a positive correlation between the serum and synovial fluid concentrations of YKL-40 [6]. Consequently, YKL-40 may be directly involved in the process of inflammation and tissue degradation in the arthritic joint. If so, measurement of YKL-40 in serum may provide new information on the arthritic disease activity compared to the more indirect measures using conventional biochemical markers, serum C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR). The aim of the present study was a more extensive and longitudinal study of variations in serum concentrations of YKL-40 in patients with RA. A comparison was performed between changes in serum YKL-40 and clinical and biochemical markers of disease activity.

Materials and methods

Patients

The study is part of two multicentre clinical trials of patients with RA [21, 22]. Study I was primarily performed to determine the bone mineral density in patients with RA and to evaluate parameters of importance for monitoring inflammatory activity in relation to bone loss in these patients [21]. Study II was performed to evaluate whether prednisolone therapy in combination with disease-modifying anti-rheumatic drug (DMARD) is superior to DMARD therapy alone in a randomized longitudinal study. Patients with clinically active RA, planned either to start or change DMARD therapy, were randomly allocated to treatment with DMARD only ($n = 38$) or in combination with prednisolone ($n = 43$). Prednisolone therapy was given as 30 mg prednisolone perorally (p.o.) daily the first week, 20 mg prednisolone p.o. daily the second week and 15 mg p.o. at day 15. The patients were then instructed to choose a prednisolone dose between 2.5 and 15 mg sufficient to control their disease activity [22]. The studies were approved by the local ethical committees of the participating centres. In accordance with the Helsinki Declaration II, each patient was informed about the study verbally and in writing, and all gave their written consent. The patients were informed of the possibility of withdrawing from the study at any time.

The study included 156 patients (75 patients from study I and 81 from study II; 125 women and 31 men, aged 20–83 yr) with RA according to the American College of Rheumatology criteria for this disease [23]. Eighty patients were in functional class I, 72 in class II and four in class III according to the Steinbrocker

classification criteria [24]. None of the patients had any known cancer, liver or lung disease at entry or had developed these diseases by August 1997 (5 yr after inclusion in the study). A total of 137 patients were treated with DMARD during the 1 yr study period: methotrexate (MTX) ($n = 49$), sulphasalazine ($n = 43$), penicillamine ($n = 21$), ercoquin ($n = 8$), gold ($n = 10$), imurel ($n = 5$), cyclosporin ($n = 1$) or no DMARD ($n = 19$). None of the patients received DMARD combination therapy. Twenty-five patients received prednisolone p.o. initially. The patients continued with this DMARD therapy throughout the study period, but the dosages were allowed to be modified accordingly to disease activity.

The patients were followed with clinical and biochemical controls at day 30, 60, 90, 120, 150, 180, 270 and 360. Patients in study II also had controls at day 1, 7 and 14. Data collected at each visit included the number of swollen joints, number of tender joints, doctor's and patient's global assessment (visual analogue scale), health assessment questionnaire (HAQ score), morning stiffness, grip strength and determination of ESR, CRP, YKL-40 and the amino-terminal propeptide of type III procollagen (PIIINP) in serum. An area-weighted swollen joint index was calculated according to Lansbury [25], where each swollen joint was multiplied by a factor weighted for the relative joint surface area: proximal interphalangeal joint ($\times 2$), metacarpophalangeal joint ($\times 5$), wrist ($\times 15$), elbow ($\times 52$), shoulder ($\times 45$), knee ($\times 104$), ankle ($\times 35$), first metatarsophalangeal joint ($\times 8$), remaining metatarsophalangeal joints ($\times 5$).

The patients were divided into three groups at baseline according to disease activity [21]. Group I: 105 patients with clinically active and biochemically active disease defined by the presence of >2 groups of swollen joints and at least two of the following criteria: morning stiffness lasting >60 min, ESR > 35 mm in the first hour (normal range: <20 mm/h and serum CRP $\geqslant$ 150 nmol/l (normal range: <90 nmol/l). Group II: 31 patients with clinically active disease but biochemically inactive disease defined by the presence of >2 groups of swollen joints, morning stiffness lasting >60 min, ESR $\leqslant 30$ mm/h and serum CRP < 150 nmol/l. Group III: 20 patients with clinically inactive and biochemically inactive disease defined by $\leqslant 2$ swollen joints, morning stiffness lasting < 30 min, ESR $\leqslant 30$ mm/h and serum CRP < 150 nmol/l.

Biochemical analysis

Blood samples were collected between 8 a.m. and 2 p.m., allowed to clot at room temperature and then centrifuged at $2000\,g$ for 10 min. The serum and plasma samples were either analysed immediately or stored at $-80°C$ until all samples from the 1 yr study were collected (for serum YKL-40 and PIIINP analysis). Serum CRP was analysed with nephelometry. ESR, haemoglobin, leucocytes, serum alkaline phosphatase, serum aspartate aminotransferase, serum albumin and serum creatinine were determined by routine methods. PIIINP in serum was determined by radioimmunoassay

TABLE 1. Baseline demographic and clinical characteristics of patients with rheumatoid arthritis according to disease activity

	Clinically active + biochemically active	Clinically active + biochemically inactive	Clinically inactive + biochemically inactive
Number	105	31	20
Sex (female/male)	87/18	22/9	16/4
Age[a] (yr)	61 (20–83)	55 (31–79)	59 (40–75)
Disease duration (yr)	4.5 (0.5–40)	11 (0.5–40)	7.5 (0.5–44)
Swollen joints (number)	11 (4–29)	9 (4–20)	0 (0–4)
Tender joints (number)	9 (0–38)	8 (0–38)	2 (0–9)
HAQ-score (0–3)[b]	1 (0–2.88)	0.88 (0–2.13)	0.63 (0–1.25)
ESR (mm/h)	54 (2–110)	17 (4–33)	8 (3–34)
Serum CRP (nmol/l)	361 (48–1771)	90 (29–145)	35 (29–125)
Serum YKL-40 (µg/l)	234 (43–1088)	164 (58–736)	170 (136–526)

Values are medians (ranges) unless stated otherwise.
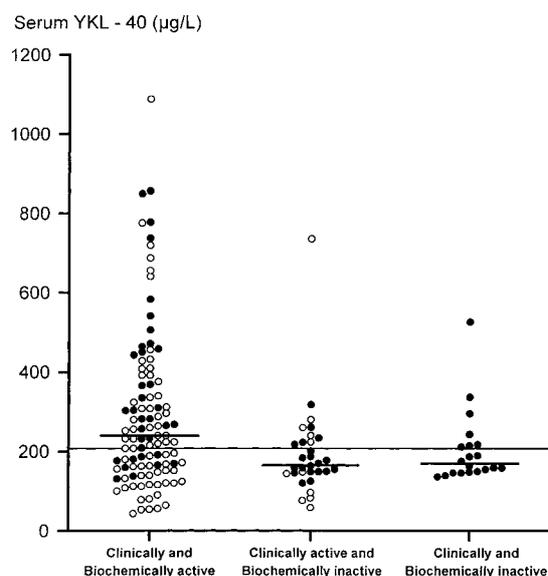[a]Mean.
[b]1 is the best.

FIG. 1. Serum YKL-40 concentrations in patients with rheumatoid arthritis according to disease activity. ●, patients from study I; ○, patients from study II. The horizontal bars show the median values of each group and the horizontal line shows the 90th percentile value (208 µg/l) of healthy age-matched controls.

(RIA) (PIIINP RIA kit, Orion Diagnostica, Finland) [26]. Serum YKL-40 was determined by RIA [6] using rabbit antibody raised against human YKL-40. Purified human YKL-40 was used for standard and tracer. The tracer was prepared by the iodogen (Pierce and Warriner, Chester Ltd, UK) method and antibody-bound and free ^{125}I-labelled YKL-40 were separated by use of Sac-Cel (a donkey anti-rabbit antibody-coated cellulose suspension; Wellcome Diagnostics Ltd, UK). The intra-assay and interassay variations were <6.5% and <12%, and the sensitivity was 20 µg/l. To eliminate the interassay variation, samples from each patient were analysed in the same assay. The median serum YKL-40 in 260 healthy adults, 116 males and 144 females, with a median age of 48 yr (range 18–79 yr) was 102 µg/l (range 38–514 µg/l, upper 90th per cent confidence

limit = 208 µg/l) [20]. There was no difference between genders ($P = 0.65$, Wilcoxon two-sample test), but a weak correlation with age (Spearman 0.30).

X-ray

Radiographs of the hands and wrist at entry and 1 yr later were evaluated blindly by the same experienced radiologist in randomly ordered groups. Each finger and wrist joint was scored according to the method of Larsen *et al.* [27], which grades joint damage on a scale from 0 (a radiologically normal joint) to 5 (a joint with maximal destruction) with reference to a standard atlas of radiographs. Progression was considered to be any magnitude of increase in Larsen score.

Statistical analysis

Statistical analysis was carried out with SPSS® (Statistical Package for the Social Sciences) Software. Because of the skewed distribution of serum YKL-40 concentrations, the results are expressed as median and range unless otherwise stated. Comparison of serum YKL-40 concentrations between groups was calculated by the non-parametric Mann–Whitney test for unpaired differences and comparison within groups by Wilcoxon's test. Correlations between the different parameters were calculated by the Spearman rho test and P values of <0.05 were considered significant. The course of the changes in serum YKL-40, serum CRP and ESR were expressed in time-integrated values, and the area under the curve (AUC) was calculated for each patient during the study using 9–12 time points (i.e. day 0, 1, 7, 14, 30, 60, 90, 120, 150, 180, 270 and 360) [28]. Pearson's test was used to evaluate whether a relationship existed between progression in Larsen score and a persistently elevated serum YKL-40, serum CRP and ESR (e.g. YKL-40 AUC > 208 µg/l, CRP AUC > 150 nmol/l and ESR AUC > 30 mm/h). To estimate the longitudinal changes in serum YKL-40 during treatment, the initial values of serum YKL-40, serum CRP and ESR were set at 100%, and values obtained after institution of treatment were expressed as a percentage of the initial value. Differences between the percentage changes in serum YKL-40, serum CRP and ESR between the two treat-

TABLE 2. Correlations between serum YKL-40, ESR and serum CRP and biochemical and clinical parameters of disease activity in patients with rheumatoid arthritis

| | Serum YKL-40/ESR/serum CRP | | | | |
| | All (n = 156) | Only patients included in study II (n = 81) | | | |
	Initial	Initial	30 days	180 days	360 days
ESR	0.42***	0.38***	0.31**	0.36**	0.46***
Serum CRP	0.38***/0.73***	−/0.51***	−/0.65***	0.27**/0.53***	0.44***/0.72***
Serum PIIINP	0.30***/0.40***/0.47***	0.35***/−/0.36***	0.44***/0.27**/0.21	0.29**/−/−	0.33**/0.38**/0.35**
Swollen joint index[a]	0.32**/0.26**/0.31**	0.35**/−/−	−/0.27**	0.32**/0.40***/0.31**	−0.36***/0.35**
Total no. of swollen joints	−/0.26***/0.37***	−/−	−/0.29**/0.36***	−/0.35***/0.34**	−0.43***/0.37**
Total no. of tender joints	−/−/0.20**	−0.33**/−/−	−0.33**/0.28**	−/−/0.29**	−/−/−

Values are Spearman's rho. **P < 0.01; ***P < 0.001; −, non-significant.
PIIINP, the amino-terminal propeptide of human type III procollagen.
[a]An area-weighted swollen joint index was calculated according to Lansbury and Haut [25], where each swollen joint is multiplied by a factor weighted for the relative joint surface area (see Materials and methods).

METHOTREXATE

Serum YKL-40 % of initial value

OTHER DMARDs

Serum YKL-40 % of initial value

METHOTREXATE

Serum CRP % of initial value

OTHER DMARDs

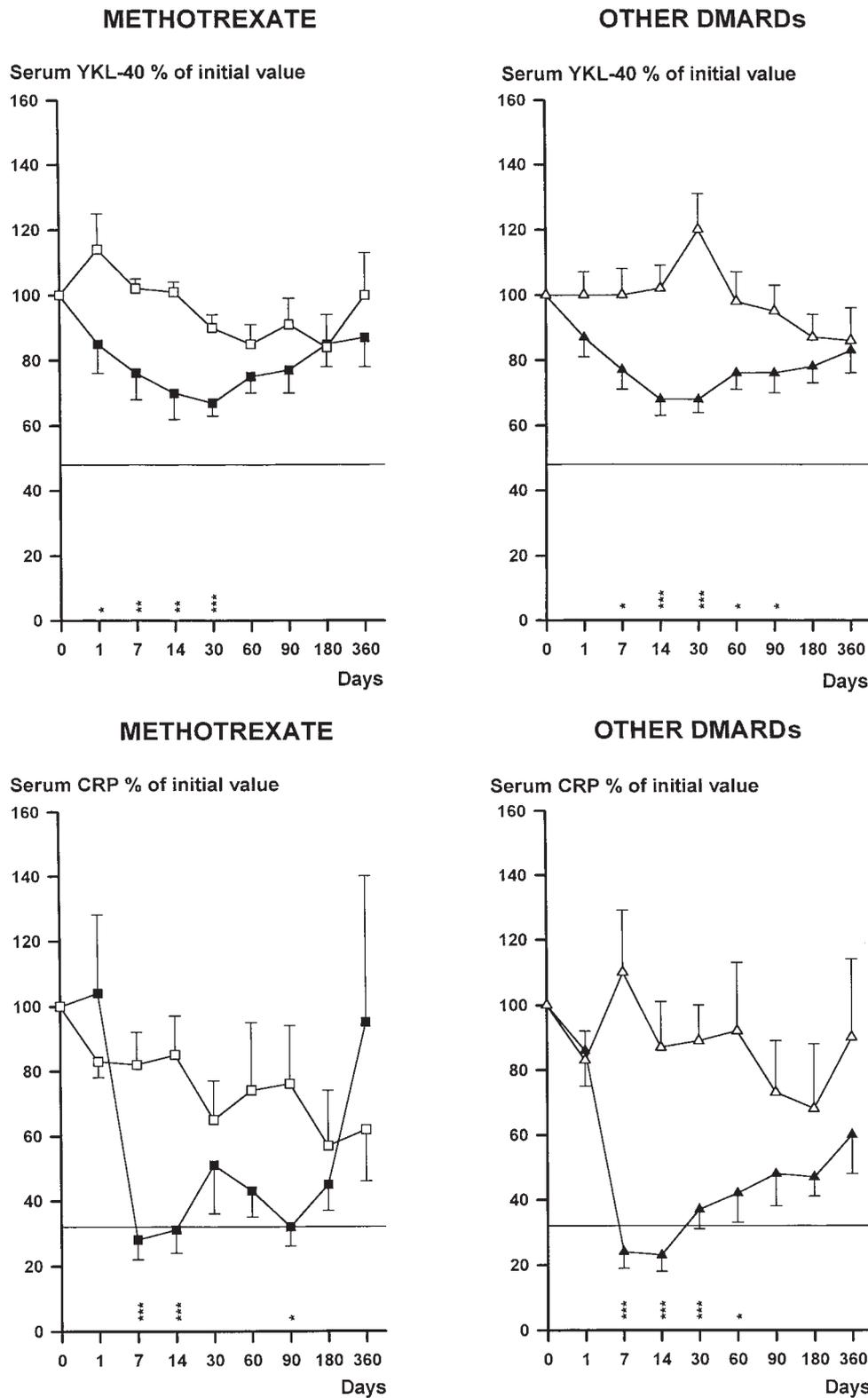### Serum CRP % of initial value

FIG. 2. Changes (expressed as a percentage of the baseline value) in serum YKL-40, serum CRP and ESR in patients with rheumatoid arthritis during treatment with DMARD only (□ △) or in combination with prednisolone (■ ▲) (30 mg prednisolone p.o. daily for 1 week, then 20 mg prednisolone p.o. daily for 1 week, 15 mg prednisolone p.o. at day 15 and thereafter further reductions in prednisolone on an individualized basis). Values are expressed as the mean ± 1 s.e.m. Student's *t*-test for unpaired values (i.e. the difference between the percentage changes in serum YKL-40 between the two groups): *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. The horizontal lines show the upper normal level.

METHOTREXATE

ESR % of initial value
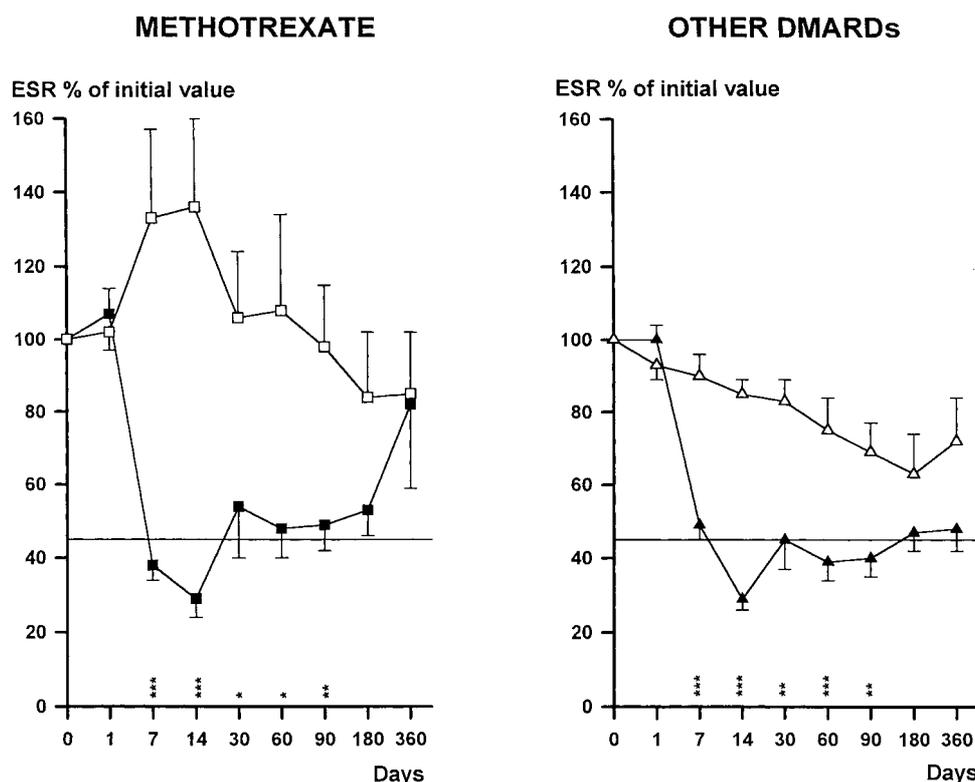
OTHER DMARDs

ESR % of initial value

FIG. 2. (*Continued*)

ment groups (study II; ± prednisolone) were estimated by Student's *t*-test for unpaired data.

Results

Serum YKL-40 in relation to disease activity

Baseline clinical and demographic features of the patients according to disease activity are presented in Table 1. The groups were similar in age, disease duration and sex ratio. Patients with clinically and biochemically active RA (Group I) had higher serum YKL-40 (median 234 μg/l, range 43–1088 μg/l) than patients with clinically active but biochemically inactive disease (164 μg/l, range 58–736 μg/l; $P = 0.0046$) (Group II), patients with clinically and biochemically inactive disease (170 μg/l, range 136–526 μg/l; $P = 0.0603$) (Group III) and age-matched controls (102 μg/l; $P < 0.01$). The individual serum YKL-40 concentrations according to disease activity are illustrated in Fig. 1. Sixty per cent of the patients in Group I had increased levels of serum YKL-40 (e.g. >208 μg/l; 90th percentile of controls) and serum YKL-40 decreased significantly (−30%, $P < 0.002$) in the patients who became clinically and biochemically inactive at 12 months ($n = 22$). Patients who remained active during the study period had unchanged serum YKL-40. Ten patients changed from inactive to active disease and they had an increase in serum YKL-40 of 50% ($P < 0.05$).

There was not always a close relationship between elevated acute-phase proteins and elevated serum YKL-40 or between normal acute-phase proteins and normal serum YKL-40 in the patients with clinically active disease (Groups I and II). Seventy-four (54%) of these 136 patients had elevated serum YKL-40 levels, 92 (68%) had elevated serum CRP and 83 (61%) had increased ESR. In 51 of these 136 patients, all three biochemical parameters were elevated, 23 patients had normal levels of all three parameters, 10 patients had normal acute-phase proteins but elevated serum YKL-40, 29 patients had elevated acute-phase proteins but normal YKL-40, 10 patients had elevated serum YKL-40 and either increased serum CRP or ESR, seven had only an elevation in ESR and six had only an elevation in serum CRP. Serum YKL-40 correlated initially (all patients) with ESR (Spearman's rho 0.42) and serum CRP (rho 0.38), but the correlation coefficients were much lower than that between ESR and CRP (rho 0.73) (Table 2). In study II, serum YKL-40 correlated with ESR and serum PIIINP throughout the study period (ESR not at day 60), and with serum CRP after day 60. During the first 6 months, serum YKL-40 correlated with the area-weighted swollen joint index, but was not related to the total number of large or small swollen joints, total number of large or small tender joints, HAQ score, grip strength, or doctor's and patient's visual analogue scale.

Serum YKL-40 in relation to progression in Larsen score

The variations in the biochemical parameters were expressed in time-integrated values during the study and

the AUC was calculated [28]. Forty-one patients were biochemically inactive throughout the 1 yr study period (i.e. normal serum CRP and ESR), and 15 of these patients had a progression in Larsen score (median progression score = 2, range 1–5). Persistently elevated serum YKL-40 (YKL-40 AUC > 208 μg/l) was found in nine of these biochemically inactive patients with clinically active disease and a progression in Larsen score.

Twenty-seven of the 50 patients with early RA (disease duration < 3 yr, 16 from study I and 34 from study II) had a progression in Larsen score after 1 yr (median progression score = 3, range 1–18). The initial concentrations of serum YKL-40, serum CRP and ESR were not related to the progression in Larsen score. By contrast, a persistently elevated serum YKL-40 (YKL-40 AUC > 208 μg/l) was related to a progression in Larsen score (Pearson's test, $P = 0.0075$). Persistently increased ESR (ESR AUC > 30 mm/h) was also related to progression in Larsen score ($P = 0.041$), whereas a persistently elevated serum CRP (CRP AUC > 50 nmol/l) was not related to a progression in Larsen score ($P = 0.70$).

Changes in serum YKL-40 during treatment with DMARD only or in combination with prednisolone (study II)

In patients treated with prednisolone in combination with MTX ($n = 18$), serum YKL-40 was 15% below initial levels after only 1 day ($P < 0.01$) and was 33% below initial levels after 30 days ($P < 0.001$). In patients treated with MTX only ($n = 17$), serum YKL-40 was reduced by 15% after 60 days ($P < 0.01$). The percentage change in serum YKL-40 between the two treatment groups was statistically significant during the first month ($P < 0.05$–$P < 0.001$) (Fig. 2). Patients treated with other DMARDs without prednisolone had a slower decrease in serum YKL-40, being statistically significant after 6 months (-13%, $P < 0.05$). Most of the patients in the prednisolone-treated group either stopped prednisolone therapy or continued with a low dose at 6 months. At this time, no difference in serum YKL-40 was found between the two treatment groups. Serum CRP was decreased by 74% and ESR by 71% ($P < 0.001$) after 14 days, and the levels in the prednisolone-treated patients were significantly lower than those in patients treated with DMARD only until day 90 (serum CRP) or day 180 (ESR) ($P < 0.05$–$P < 0.001$) (Fig. 2).

At 1 yr, 12 patients were clinically and biochemically inactive and had a decrease in serum YKL-40 of 36% ($P < 0.001$) compared to the initial values. None of these patients had an elevated serum YKL-40 at 12 months.

Discussion

Chronic active inflammation of the rheumatoid joint often leads to irreversible destruction of articular cartilage and subchondral bone. Measurements of the acute-phase response, serum CRP and ESR, are considered to be suitable biochemical markers for long-term monitoring of disease activity in RA [29–31]. Radiological progression, including loss of joint space, bone erosions or subchondral cysts, represents one of the outcome measures in RA and the progression in the small hand joints parallels the radiological changes in other joints [32]. However, differences exist between clinical disease activity and the level of ESR or serum CRP. Normal values of ESR and CRP are found in patients with apparently clinically active disease [29, 31], and the development of bone erosions in the hands from patients with early RA can occur independently of clinical symptoms and the acute-phase response [33]. Additional biochemical markers of disease activity are therefore needed.

Our present findings confirm that serum YKL-40 is influenced by the disease activity in RA as evaluated by clinical markers of disease activity as well as by ESR and CRP. Serum YKL-40 was increased in 54% of the patients with clinically active RA. Patients with clinically active disease who became inactive during the study had a significant decrease in serum YKL-40, whereas patients who remained clinically active had unchanged serum YKL-40 levels. Patients changing from clinically inactive to active disease showed an increase in serum YKL-40. Furthermore, serum YKL-40 had a predictive value for the risk of radiological progression of joint damage in patients with early RA.

In some patients, serum YKL-40 provided information on disease activity different from that of ESR and serum CRP. An elevated serum concentration of YKL-40 was observed in some patients showing radiological progression in Larsen score, but with normal levels of the acute-phase reactants, and normal serum YKL-40 levels were observed in some patients with biochemically and clinically active disease. The decrease in serum YKL-40 in patients treated with DMARD in combination with prednisolone was much lower compared to that observed in the acute-phase proteins. Low, albeit significant, correlations were found between serum YKL-40 and ESR and serum CRP, but less significant than the correlation between ESR and serum CRP. YKL-40 also correlated with the area-corrected swollen joint score and serum PIIINP, a biochemical marker of the formation of type III collagen as part of synovial inflammation [34, 35].

Our results indicate that serum YKL-40 may reflect a different and more local aspect of the inflammatory process than serum CRP and ESR in patients with RA. The acute-phase proteins are not secreted by the cells in an arthritic joint, but their circulating levels represent a non-specific distant response by hepatocytes to mediators like interleukin-6, tumour necrosis factor and interleukin-1β [36, 37]. YKL-40 is not secreted by hepatocytes [19] and a plausible explanation of the elevated serum YKL-40 levels in patients with active RA would be that the protein is secreted in excess in the arthritic joint. This assumption is supported by the findings in our previous study [6]. We observed a 10-fold

higher concentration of YKL-40 in synovial fluid compared to serum in patients with RA, as well as a positive relationship between YKL-40 levels in serum and synovial fluid. YKL-40 can originate from articular chondrocytes [5, 7], synovial cells (probably activated macrophage or macrophage-like cells in the inflamed synovial membrane) [13, 14] or from exocytosis of YKL-40 in the specific granules of neutrophils [15] found in large amounts in the synovial fluid and in the cartilage–pannus junction [38–40]. However, our present study cannot determine which of these cell types are the major producers of YKL-40 in an arthritic joint.

The YKL-40 gene has been sequenced, but promoter analysis has not been described [17]. The regulation of YKL-40 is little studied; transforming growth factor-β has been shown to reduce the level of YKL-40 mRNA in chondrocytes to a barely detectable level [5], whereas the expression was not influenced by insulin-like growth factor-1 in chondrocytes [5] or by interleukin-1 and tumour necrosis factor-α in synovial cells [13]. We found that serum YKL-40 in patients with active RA had already decreased significantly after 1 day of prednisolone therapy, indicating that steroids may have a direct effect on the expression of YKL-40.

The present study indicates that serum YKL-40 varies according to variations in disease activity in RA as estimated by clinical signs and acute-phase proteins. Our previous findings are consistent with a local release of YKL-40 in the arthritic joints followed by an increase in serum YKL-40. Some of our observations in the present study suggest that YKL-40 may provide information on disease activity different from that of the conventional markers. YKL-40 may prove to be a new tool for the estimation of disease activity and study of the pathophysiology of RA.

Acknowledgements

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