Clinical Significance of Cartilage Biomarkers for Monitoring Structural Joint Damage in Rheumatoid Arthritis Patients Treated with Anti-TNF Therapy

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Abstract

Purpose: With the current use of biologics in rheumatoid arthritis (RA), there is a need to monitor ongoing structural joint damage due to the dissociation of articular cartilage damage from disease activity of RA. This study longitudinally analyzed levels of serum cartilage biomarkers during 54 weeks of infliximab therapy, to evaluate the feasibility of biomarkers for monitoring structural joint damage.

Methods: Subjects comprised 33 patients with early RA and 33 patients with established RA. All patients received 3 mg/kg of infliximab and methotrexate for 54 weeks. Levels of the following serum cartilage markers were measured at baseline and at weeks 14, 22, and 54: hyaluronan (HA); cartilage oligometric matrix protein (COMP); type II collagen (CII)-related neoepitope (C2C); type II procollagen carboxy-propeptide (CPII); and keratin sulfate (KS). Time courses for each biomarker were assessed, and relationships between these biomarkers and clinical or radiographic parameters generally used for RA were investigated.

Results: Levels of CRP, MMP-3, DAS28-CRP, and annual progression of TSS were improved to similar degrees in both groups at week 54. HA and C2C/CPII were significantly decreased compared to baseline in the early RA group ($p<0.001$), whereas HA and COMP, but not C2C/CPII, were decreased in the established RA group. Strikingly, serum C2C/CPII levels were universally improved in early RA, regardless of EULAR response grade. Both ΔHA and ΔC2C/CPII from baseline to week 54 correlated significantly with not only ΔCRP, but also ΔDAS28 in early RA. Interestingly, when partial correlation coefficients were calculated by standardizing CRP levels, the significant correlation of ΔHA to ΔDAS28 disappeared, whereas correlations of ΔC2C/CPII to ΔDAS28, ΔJNS, and ΔHAQ remained significant. These results suggest a role of ΔC2C/CPII as a marker of ongoing structural joint damage with the least association with CRP, and that irreversible cartilage damage in established RA limits restoration of the C2C/CPII level, even with tight control of joint inflammation.

Conclusion: The temporal course of C2C/CPII level during anti-TNF therapy indicates that CII turnover shifts toward CII synthesis in early RA, but not in established RA, potentially due to irreversible cartilage damage. ΔC2C/CPII appears to offer a useful marker reflecting ongoing structural joint damage, dissociated from inflammatory indices such as CRP and MMP-3.

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Introduction

Anti-tumor necrosis factor (TNF) therapy is considered the global standard in the treatment of rheumatoid arthritis (RA), originally with the purpose of achieving clinical remission and now extending to structural remission at the radiographic level. Mounting evidence has accumulated that anti-TNF therapy not only inhibits radiographic progression of joint space narrowing, but also promotes joint space widening, particularly in patients with early RA, in whom annual changes in total modified van der Heijde (vdH)-Sharp score (TSS) are negative [1,2]. These observations allow clinicians to expect that TNF-blockade is capable of regenerating cartilage. However, 2-dimensional radiographic assessments based on TSS have not yet confirmed whether ongoing cartilage damage can be precisely evaluated. Ultrasonography and magnetic resonance imaging have recently been reported to allow detection of subclinical joint damage in patients showing clinical remission, suggesting a dissociation between clinical remission and structural joint deterioration [2,3]. Alternative tools that can assess ongoing joint destruction more easily than these imaging modalities should facilitate the evaluation of anti-rheumatic therapy with the potential to target structural remission. Molecular-marker technology (i.e., biomarkers) reportedly offer

Table 1. Baseline characteristics of the patients with early and established RA enrolled in this study*.

	Early RA (<9 months)	Established RA (>10 yrs)
No. of patients	33	33
Mean age	46.2 (19–75)	55.6 (34–80)
Gender (male/female)	10/23	6/27
Disease duration [months]	5.5 (2–9)	285 (122–516)
Swollen joint counts	10.3 (3–25)	10.3 (0–23)
Tender joint counts	8.8 (1–24)	8.6 (0–27)
CRP [mg/dl]	4.3 (0.2–11.0)	3.2 (0.1–10.9)
MMP-3 [ng/ml]	367 (31–1378)	302 (37–1292)
Rate of anti-CCP antibody [%]	82	85
DAS28-CRP	5.24 (3.11–7.75)	4.8 (2.54–6.83)
HAQ score	1.68 (0.75–2.38)	2.12 (0.75–3.00)
corticosteroid administration [%(cases)]	9 (3)	18 (6)

*Except where indicated otherwise, values are expressed as the mean (range).
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greater reliability and sensitivity than 2-dimensional radiography in clinical applications [4–6] and may offer a potential alternative to evaluate ongoing cartilage destruction in RA.

Alteration of articular cartilage turnover under arthritic conditions finally depends on the balance between the synthesis and degradation of cartilage matrix [7,8]. This can be monitored by measuring cartilage-derived synthesis and degradation molecules released into biological fluids, such as synovial fluid, serum and urine. These cartilage-derived biomarkers have been shown to reflect structural joint damage in RA and allow assessment of therapeutic efficacy in candidate anti-rheumatoid therapy. Existing biomarkers include cartilage oligometric matrix protein (COMP), human cartilage glycoprotein-39 (YKL-40), type II collagen (CII)-related neoepitope (C2C), carboxy-terminus of three-quarter peptide from cleavage of type I collagen and CII (C1,2C), type II procollagen carboxy-propeptide (CPII), C-telopeptide of type II collagen (CTX-II), keratin sulfate (KS-5D4), and aggrecan neoepitope (CS-846). Although controversy remains about which of the biological fluids offers the best sampling source and about diurnal and activity-related variations in each biomarker [9], a fundamental principle is that markers for cartilage degradation generally increase with the progression of joint destruction, whereas markers for cartilage synthesis increase following successful treatment with anti-TNF therapy [10]. The current use of biologics in RA makes it increasingly important to identify useful and simple blood tests that can precisely reflect responses to treatment, particularly in terms of cartilage turnover and systemic inflammation resulting from RA.

Despite the advantages of technical simplicity, the practical application of serum cartilage-derived biomarkers to date has remained limited. This is due, in part, to the fact that superiority over traditional laboratory markers has not been studied in a longitudinal fashion. The present study analyzed time courses for serum levels of cartilage markers during 54 weeks of infliximab therapy in two different cohorts of early and established RA, and compared the results with other laboratory, clinical and radiographic parameters generally used for RA. This study also estimated the feasibility of using cartilage biomarkers as a potential indicator of structural joint deterioration in RA.

Materials and Methods

All study protocols were approved by the institutional review board at Saitama Medical Center. All participants were informed about the goals and methods of the study and written consent was obtained prior to enrolment.

Patients

In this study, a total of 66 patients were enrolled from the Division of Rheumatology and Clinical Immunology at Saitama Medical Center, and all patients fulfilled the diagnostic criteria for RA according to American College of Rheumatology criteria [11]. Thirty-three patients with arthritis symptoms of <9 months duration were classified as having early RA, and 33 patients with disease duration >10 years were classified as showing established RA. Baseline characteristics of patients are shown in Table 1. All patients had clinically active disease, despite administration of conventional first-level disease-modifying anti-rheumatic drugs, and the mean 28-joint disease activity score (DAS28)-CRP at baseline was 5.24 for early RA and 4.8 for established RA. The rate of anti-cyclic citrullinated peptide (anti-CCP) antibody was 82% (27 patients) for the early RA group and 85% (28 patients) for the established RA group. Infliximab was administered at 3 mg/kg dose in weeks 0, 2, and 6, and then every 8 weeks. MTX was concomitantly administered at 6–10 mg/week in all patients. Patients were allowed to continue use of non-steroidal anti-inflammatory drugs and oral glucocorticoids (prednisolone-equivalents <10 mg/day) that they had been taking at study entry.

Patients were evaluated for therapeutic response at baseline and 14, 22, and 54 weeks after starting infliximab. At each evaluation, blood samples were obtained and sera were separated and stored at −80°C until needed for biomarker analysis.

Clinical evaluation of therapeutic response

The following clinical and laboratory parameters were longitudinally examined in each patient: CRP; stromelysin 1 (MMP-3); modified Health Assessment Questionnaire (HAQ) score; and DAS28-CRP. Scores for DAS28-CRP are reportedly lower than the original DAS28 assessments using the erythrocyte sedimentation rate [12] and were defined as follows: ≥4.1, high activity; ≥2.7 to <4.1, moderate activity; ≥2.3 to <2.7, low activity; and <2.3, remission. In terms of radiographic analysis, radiographs of

both hands and feet at baseline and 54 weeks were available for 26 patients in the early RA group and 23 patients in the established RA group. Two expert readers independently scored articular damage and progression in a blinded fashion according to the modified vdH-Sharp scoring method. Progression of TSS from baseline to week 54 (ΔTSS) was determined, and the proportion of patients with ΔTSS≤0 was calculated.

Cartilage biomarker analyses

The neoepitope resulting from collagenase cleavage of CII (i.e., C2C) and the c-propeptide cleaved from procollagen type II (i.e., CPII) were used as indicators of the degradation and synthesis of CII, respectively. Serum levels of each marker were measured using enzyme-linked immunosorbent assay (ELISA) (IBEX Technologies, Montreal, Quebec, Canada). The ratio C2C/CPII was used as an indicator of CII turnover, as previously reported [13,14]. Serum COMP levels were determined by sandwich ELISA (BioVendor Laboratory, Brno, Czech Republic), using 2 monoclonal antibodies against separate antigenic determinants of human COMP molecules. Serum HA levels were determined using an HA Assay Kit (IBA method; Seikagaku, Tokyo, Japan) utilizing HA-binding protein. KS was determined by high-performance liquid chromatography (HPLC) after digestion with keratanase II (Seikagaku), as reported previously [15,16]. Serum samples were treated with actinase E (Kaken Pharmaceutical, Tokyo, Japan) and the negatively charged substance containing KS was fractionated by Q sepharose and digested by keratanase II. These sequential enzymatic digestions yielded KS-derived β-galactosyl-(1–4)-6-O-sulfo-N-acetylglucosamine (m-ks) and β-6-O-sulfo-garactosyl-(1–4)-6-O-sulfo-N-acetylglucosamine (d-ks), which were measured using HPLC. Total KS was calculated as the sum of m-ks and d-ks values.

Statistical analysis

Analysis of our data revealed that most of the clinical, radiographic, and laboratory results were non-parametric. Statistical comparisons of laboratory parameters or cartilage biomarker levels at each time point with those at baseline were performed using Wilcoxon's matched-pairs signed-ranks test (two-tailed). Spearman's rank correlation coefficient was used to analyze relationships between changes in individual biomarkers and changes in laboratory or functional or radiographic parameters of RA. To remove the effects of decreased inflammation (i.e., CRP level) resulting from anti-TNF therapy on cartilage turnover, partial correlation coefficients controlling for CRP level were calculated to examine the relationship between cartilage biomarkers and measures of RA disease activity. Subgroup analysis was conducted based on European League of Associations for Rheumatology (EULAR) response criteria, such as good response, moderate response, and no response. As an indicator of CII turnover, C2C/CPII ratios in each response group were analyzed longitudinally, and changes from baseline to week 54 (i.e., C2C/CPII improvement) were compared between the three subgroups using the Kruskal-Wallis test. Statistical analyses were performed using SPSS version 17.0 software (SPSS, Chicago, IL). Values of $p<0.05$ were considered significant.

Sample size analysis for Wilcoxon's signed-ranks test was performed to demonstrate differences between serum level at baseline and at week 54 under the effect size given in each biomarker or laboratory index. In post-hoc analysis for early RA, 11, 60, 8, 5, 13, and 22 patients would be required to demonstrate a difference with an alpha level of 0.05 and 80% power, for C2C/CPII, HA, CRP, DAS28, MMP3, and HAQ, respectively. Similarly, for established RA, 29, 30, 20, 13, 7, and 18 patients would be required to demonstrate a difference, with an alpha level of 0.05 and 80% power, for KS, HA, COMP, CRP, DAS28, and MMP3, respectively. Sample sizes for correlation analysis were also analyzed to detect a moderate to large correlation coefficient ($r>0.4$) that was significantly different from the presence of no correlation ($r = 0$) with an alpha level of 0.05 and 80% power. In post-hoc analysis for early RA at week 54, 29 and 26 patients would be required to represent a given bivariate correlation coefficient with 80% power for ΔC2C/ΔCPII vs. ΔCRP and ΔC2C/ΔCPII vs. ΔDAS28, respectively. Similarly, 31 patients would be required to detect a given partial correlation coefficient with 80% power for ΔC2C/ΔCPII vs. ΔDAS28. In the established RA at week 54, 27 and 20 patients would be required to represent a given bivariate correlation coefficient with 80% power for ΔC2C/ΔCPII vs. ΔJNS and ΔC2C/ΔCPII vs. ΔHAQ, respectively. Regarding the partial correlation coefficient, 31 patients each would be required both for ΔC2C/ΔCPII vs. ΔJNS and for ΔC2C/ΔCPII vs. ΔHAQ. Taken together with these data, the projected sample size offering sufficient statistical power was 30 patients each in the early and established RA groups.

Results

Clinical evaluation

Of the 33 patients in the early RA group, 1 patient achieved clinical remission and 1 patient exhibited secondary loss of efficacy after 6-month infliximab therapy. These 2 patients discontinued infliximab, and the latter patient switched to tocilizumab. One patient experienced anaphylactic reaction at week 38 and switched to etanercept. Overall, 3 patients withdrew from the study, and the remaining 30 patients in the early RA group completed 54 weeks of infliximab therapy. In the established RA group, 5 patients exhibited secondary loss of efficacy and switched to etanercept ($n = 3$) or tocilizumab ($n = 2$). One patient discontinued infliximab at week 22, because she was planning to become pregnant. Overall, 6 patients were excluded and the remaining 27 patients in the established RA group completed 54 weeks of infliximab therapy.

As expected, laboratory indices for RA disease activity, such as CRP, MMP-3 and DAS28-CRP, had decreased significantly by week 54 in both groups (Table 2). The decrease in DAS28-CRP was prominent in patients with early RA, with mean score at week 54 below the level of clinical remission. Mean HAQ score was significantly decreased at week 54 in the early RA group, but remained unchanged in the established RA group. When DAS28-CRP scores were assessed using EULAR response criteria, 90% and 78% of patients were categorized as showing good or moderate response in the early and established RA groups, respectively, with no significant difference apparent between groups. Radiographic structural assessment using the TSS revealed that mean ΔTSS per year (annual progression) was 3.7 in the early RA group and 4.0 in the established RA group, while the proportion with ΔTSS≤0 exceeded 70% in both groups, suggesting that our clinical study using infliximab yielded successful clinical results comparable to those in a previous study in Japan [17].

Temporal changes in cartilage biomarkers during 54-week infliximab therapy

In the early RA group, serum levels of HA and C2C/CPII gradually decreased over time during 54-week infliximab therapy, and levels of HA at weeks 14, 22 and 54, and C2C/CPII at weeks 22 and 54 were significantly lower than each baseline level ($p<0.001$). These two biomarkers appeared to synchronize with

Table 2. Time-course changes in biochemical, clinical, radiographic, and functional measures during 1-year infliximab therapy.

	Time after starting infliximab			
	0W (baseline)	**14W**	**22W**	**54W**
Early RA (n = 30)				
CRP [mg/dl]	4.12†	1.43**	1.02**	0.45**
DAS28-CRP	5.16	3.13**	2.74**	2.2**
MMP-3 [ng/ml]	342	167	116*	105*
HAQ score†	1.46	0.92**	0.9**	0.8**
TSS (SD) (n = 26)	10.5 (18.7)	n.d.***	n.d.	14.2 (20.1)
JNS (SD) (n = 26)	4.8 (7.6)	n.d.	n.d.	7.2 (10.3)
ΔTSS (mean/median)				3.7/0
Rate of ΔTSS≤0 [%(cases)]				73 (19)
EULAR category of response [% (cases)]				
Good				63 (19)
Moderate				27 (8)
No response				10 (3)
Established RA (n = 27)				
CRP [mg/dl]	2.91	0.68**	0.66*	0.66*
DAS28-CRP	5.11	2.96**	2.76**	2.80**
MMP-3 [ng/ml]	298	92	98*	91*
HAQ score	1.88	1.7	1.71	1.73
TSS (SD) (n = 23)	211.2 (90.2)	n.d.	n.d.	215.4 (96.3)
JNS (SD) (n = 23)	85.8 (43.6)	n.d.	n.d.	88.1 (44.2)
ΔTSS (mean/median)				4.0/0
Rate of ΔTSS≤0 [%(cases)]				70 (16)
EULAR category of response [% (cases)]				
Good				41 (11)
Moderate				37 (10)
No response				22 (6)

†Except where indicated otherwise, values are expressed as the mean.
*$p<0.05$ versus baseline levels.
**$p<0.001$ versus baseline levels.
***n.d., not determined.
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decreasing CRP level over the 54 weeks of infliximab therapy. In contrast, COMP level remained constant during infliximab therapy (Table 3, Fig. 1A). Serum KS level slightly increased at week 14, followed by a gradual decrease to the baseline level at week 54. In the established RA group, serum level of HA was significantly decreased at week 14 ($p<0.05$) and became constant, demonstrating a quite similar pattern to that of CRP, whereas C2C/CPII remained unchanged during 54 weeks (Table 3, Fig. 1B). Level of serum COMP in established RA, which demonstrated a higher baseline level than in early RA, gradually decreased during the 54-week infliximab therapy with significant differences at week 54 ($p<0.05$). In contrast, level of serum KS in established RA, which also demonstrated a higher baseline level than in early RA, gradually increased with significant differences at weeks 22 and 54 compared to baseline ($p<0.05$).

Correlations between cartilage biomarkers and RA disease activity markers

Correlations between levels of cartilage biomarkers and degree of RA disease activity (e.g., CRP, MMP-3, and DAS-28), radiographic progression (e.g., ΔJNS) and patient function (e.g., HAQ score) were investigated at weeks 22 and 54. Several marker pairs with significant correlations are summarized in Table 4. Among the four cartilage biomarkers tested, only C2C/CPII and HA level yielded strong linear correlations with several disease activity measures of RA. Since the degree of structural joint damage, particularly in terms of cartilage destruction, is reportedly dissociated from the degree of joint inflammation, the present analysis focused on whether temporal changes in cartilage turnover were associated with the degree of CRP decrement. In the early RA group, ΔC2C/ΔCPII and ΔHA displayed significant correlations with ΔCRP at both weeks 22 and 54. Correlation with ΔDAS28 was observed at week 22 for ΔHA, and at both weeks 22 and 54 for ΔC2C/ΔCPII. Interestingly, according to partial correlation coefficients, the significant correlation between ΔHA and ΔDAS28 disappeared when the level of CRP was standardized. In contrast, the significant correlation between ΔC2C/ΔCPII and ΔDAS28 remained present even after standardization of CRP levels. In the established RA group, ΔC2C/ΔCPII correlated with neither ΔCRP nor ΔDAS28, whereas ΔHA did correlate with ΔCRP at both weeks 22 and 54. Of note is the finding that ΔC2C/ΔCPII significantly correlated with ΔJNS and

Table 3. Time-course changes in the levels of cartilage biomarkers during 1-year infliximab therapy.

	Time after starting infliximab			
	0W (baseline)	14W	22W	54W
Early RA (n = 30)				
HA [ng/ml]	420 (923) †	306 (852)*	134 (166)*	81 (69)**
KS [μg/ml]	0.87 (0.30)	0.96 (0.37)*	0.90 (0.31)	0.85 (0.22)
COMP [ng/ml]	545 (297)	549 (237)	561 (232)	570 (239)
C2C [ng/ml]	229 (47)	204 (45)	171 (46)*	156 (46)**
CPII [ng/ml]	733 (304)	858 (437)	875 (416)*	997 (489)*
C2C/CPII	0.34 (0.17)	0.32 (0.16)	0.20 (0.04)**	0.17 (0.05)**
Established RA (n = 27)				
HA [ng/ml]	335 (301)	199 (209)*	191 (196)*	193 (199)*
KS [μg/ml]	1.05 (0.34)	1.12 (0.43)	1.22 (0.38)*	1.25 (0.46)*
COMP [ng/ml]	845 (321)	788 (278)	734 (267)	669 (230)*
C2C [ng/ml]	224 (68)	231 (62)	211 (58)	264 (54)
CPII [ng/ml]	1039 (465)	1087 (439)	834 (306)	886 (243)*
C2C/CPII	0.28 (0.15)	0.27 (0.13)	0.3 (0.11)	0.31 (0.12)

†Values are expressed as mean (SD).
*p<0.05 versus baseline levels.
**p<0.001 versus baseline levels.
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ΔHAQ at week 54, and these significant correlations were present even after standardizing CRP level. These results suggest that ΔHA preferentially correlated with the level of CRP, while ΔC2C/ΔCPII represented a CRP-independent indicator of joint destruction reflecting radiographic joint space narrowing and patient function.

Association between balance of CII synthesis/degradation and efficacy of infliximab

As C2C/CPII preferentially reflected joint damage independent of changes in inflammatory indices, C2C/CPII was further analyzed for relationships with EULAR response grade after 54 weeks of infliximab therapy. Strikingly, in the early RA group, C2C/CPII was reduced (i.e., improved), regardless of responsiveness to infliximab, indicating that even in non-responders, the balance of CII synthesis/degradation became shifted toward synthesis (Fig. 2A). By contrast, C2C/CPII in the established RA group universally increased (i.e., worsened), regardless of responsiveness to infliximab, indicating that the net balance of CII synthesis/degradation was shifted toward degradation even in good responders (Fig. 2B). For all patients, C2C/CPII in non-responders was increased (i.e., worsened) compared to baseline, whereas C2C/CPII in moderate or good responders was reduced (i.e., improved) from baseline (Fig. 2C).

Discussion

RA is an inflammatory joint disease that predominantly involves the synovial tissues of joints and is characterized by variable disease onset and clinical course, ultimately resulting in structural joint destruction and subsequent physical disability. Early treatment with anti-TNF therapy is currently accepted as an effective strategy to achieve clinical and structural remission, potentially improving physical disability. In the present study, 54-week treatment with infliximab achieved satisfactory results according to the levels of CRP, MMP-3, and DAS28, EULAR response criteria, and the rate of ΔTSS≤0. Although these clinical measures for RA were similarly improved in both early and established RA, C2C/CPII as an indicator of CII turnover was significantly improved from baseline in early RA, but not in established RA. Strikingly, C2C/CPII was universally improved and shifted toward CII regeneration in early RA, regardless of EULAR response grade. In contrast, C2C/CPII was universally shifted toward CII degradation in established RA, regardless of

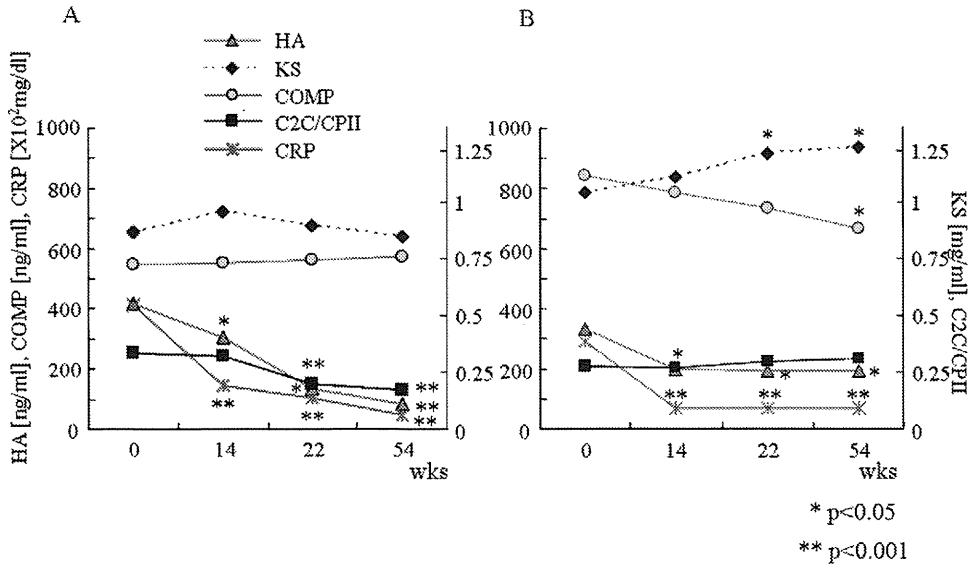

Figure 1. Temporal course of cartilage biomarker levels during 54-week infliximab therapy. Data for each time point represent mean levels of serum CRP, HA, COMP, KS, and C2C/CPII in early RA (A) and established RA (B). Standard deviation (SD) error bars are not plotted in these graphs for clarity and are shown in Table 3. Statistical analyses were performed using Wilcoxon's matched-pairs signed-ranks test, two-tailed. *p<0.05 versus level at baseline. **p<0.001 versus level at baseline.
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Table 4. Spearman's correlation coefficients and partial correlation coefficients of cartilage markers vesus RA disease markers.

		22W		54W	
		Spearman's r	**partial r****	**Spearman's r**	**partial r**
Early RA					
ΔC2C/ΔCPII	vs. ΔCRP	0.44 (0.02)*	n.a.¶	0.50 (0.01)	n.a.
	vs. ΔDAS28	0.49 (0.03)	0.48 (0.04)	0.52 (0.01)	0.49 (0.02)
	vs. ΔKS	n.s.	n.s.†	−0.46 (0.03)	n.s.
ΔHA	vs. ΔCRP	0.37 (0.04)	n.a.	0.45 (0.03)	n.a.
	vs. ΔDAS28	0.52 (0.02)	n.s.	0.43 (0.04)	n.s.
	vs. ΔMMP-3	0.63 (0.02)	n.s.	0.74 (0.002)	0.48 (0.04)
Established RA					
ΔC2C/ΔCPII	vs. ΔCRP	n.s.	n.a.	n.s.	n.a.
	vs. ΔDAS28	n.s.	n.s.	n.s.	n.s.
	vs. ΔKS	n.s.	n.s.	−0.49(0.03)	n.s.
	vs.ΔJNS	n.d.	n.d.‡	0.51 (0.03)	0.48 (0.04)
	vs.ΔHAQ	n.d.	n.d.	0.58 (0.02)	0.49 (0.03)
ΔHA	vs. ΔCRP	0.56 (0.02)	n.a.	0.43 (0.04)	n.a.
	vs. ΔDAS28	n.s.	n.s.	n.s.	n.s.
	vs. ΔMMP-3	0.49 (0.04)	n.s.	0.51 (0.04)	n.s.

*Values are correlation coefficients calculated using Spearman's rank correlation. P values are expressed in parentheses. $p<0.05$ is considered as statistically significant.
**Partial correlation coefficients were obtained after controlling CRP level for each marker pair.
¶n.a., not applicable.
†n.s., not significant.
‡n.d., not determined.
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EULAR response grade. From the perspective of CII turnover, anti-TNF therapy should clearly be initiated while the patient is still in the early phase, while the regenerative capacity of articular cartilage is maintained and before irreversible structural joint damage occurs. Past clinical trials, such as the Best study, have demonstrated that patients initially treated with infliximab exhibited persistent low disease activity even after the cessation of infliximab, suggesting the clinical significance of early introduction of aggressive treatment in early RA with poor prognostic factors [18].

A noteworthy finding was that annual changes in cartilage biomarker levels correlated with annual progression of joint destruction and physical function after anti-TNF therapy. To the best of our knowledge, no previous studies have provided such insights. Significant correlations were found between ΔC2C/CPII and ΔHAQ ($r=0.58$, $p=0.02$) and between ΔC2C/CPII and ΔJNS ($r=0.51$, $p=0.03$) in our established RA cohort. The fact that joint space narrowing on radiography largely reflects loss of cartilage rather than bony erosion may explain the close relationship between ΔJNS and ΔC2C/CPII. Although determining whether HAQ improvement is a cause or consequence of decreased ΔC2C/CPII is difficult, one potential explanation for this correlation is that high activity and subsequent mechanical

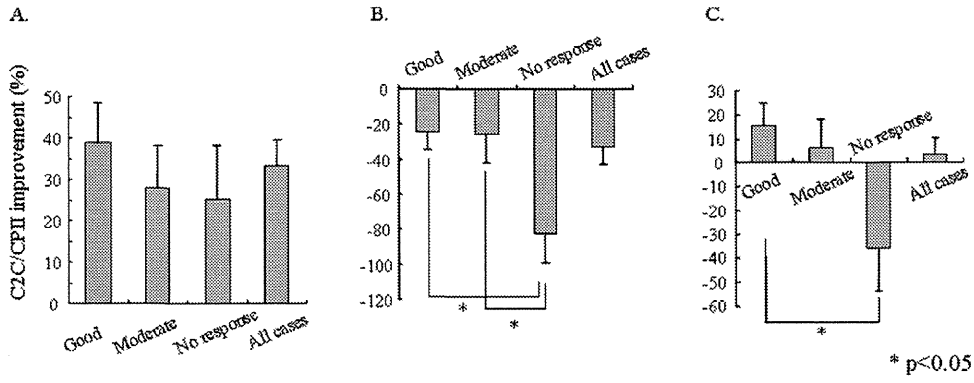

Figure 2. Improvement of C2C/CPII from baseline to week 54 was assessed in early RA (A), established RA (B), and all patients (C). Data are expressed as mean (± SD) percentage of baseline. Patients were divided into three subgroups according to the degree of clinical response at week 54 using EULAR response criteria. Positive values signify that the balance of CII synthesis/degradation is biased toward synthesis, while negative values indicate that the balance is biased toward degradation. Statistical analysis was performed using the Kruskal-Wallis test. *$p<0.05$.
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loading on cartilage either resulted in or is attributed to improved CII turnover, as reported by Roos et al. [19].

Serum levels of KS have been reported as an indirect measure of aggrecan turnover in articular cartilage and further analyzed for a role as a predisposing factor for osteoarthritis (OA) with a polyarticular, progressive phenotype [20]. KS level is elevated not only in patients with cartilage degeneration, but also in healthy individuals with higher sports activity [21], indicating that KS level can universally elevate in cases with increased cartilage turnover, even in normal cartilage. Wakitani et al. have reported that serum KS level in the knee OA is elevated more in the early stage than in the advanced stage, suggesting that KS reflects aggrecan turnover rather than the degree of joint destruction [16]. Similarly, levels of serum CS-846 epitope, as the marker for newly synthesized aggrecan, have been shown to increase in slowly progressive RA and signify an ability or attempt to repair damaged cartilage matrix [22,23]. From this perspective, gradually increased KS turnover in established RA was potentially attributable to not only persistent aggrecan release from cartilage, but also the fact that newly synthesized aggrecan cannot be incorporated into cartilage matrix that has been inherently damaged at baseline. In cases of early RA, KS levels were increased in week 14, but stabilized thereafter due to the inhibitory effects of TNF blockade on cartilage degradation, leading to normalization of cartilage turnover.

Contrasting results were obtained regarding the temporal course of serum COMP levels between early and established RA. Numerous studies have proposed the feasibility of serum COMP levels in monitoring articular cartilage damage or predicting the efficacy of anti-TNF therapy in RA [24–26]. In our established RA cohort, serum COMP levels were high at baseline, and gradually decreased during the course of infliximab therapy, as previously reported [24]. However, in early RA, serum COMP levels at baseline were low, and remained unchanged over 54-week infliximab therapy, despite fully exertion of the therapeutic effects of infliximab. Given the evidence that serum COMP levels elevate with increasing physical activity [27], constant levels of COMP over time in early RA might theoretically be explained if the decrement in COMP levels induced by infliximab is balanced by increased physical activity as evidenced from decreased HAQ scores.

Most measures of RA disease activity, such as the simplified disease activity index, Boolean criteria, and DAS28, exhibit correlations with CRP, because CRP is involved in each definition. As for cartilage biomarkers, this study showed that ΔHA and ΔC2C/CPII correlated significantly with not only ΔCRP, but also ΔDAS28 in early RA. Interestingly, when partial correlation coefficients were calculated by standardizing CRP levels, the significant correlation of ΔHA with ΔDAS28 disappeared, whereas correlations of ΔC2C/CPII with ΔDAS28, ΔJNS, and ΔHAQ were still significant. These results suggest a role of ΔC2C/CPII as a marker of ongoing structural joint damage with the least association to markers for systemic inflammation, such as CRP and erythrocyte sedimentation rate. Indeed, serum cytokine profile among the patients with established RA in this study revealed that levels of most inflammatory cytokines, including IL-6, TNF, and IL-17, were decreasing with decreasing CRP level over 54-week of infliximab therapy, whereas C2C/CPII level deteriorated over time (unpublished data, Fig. S1).

Significant concerns remain as to the differences in cartilage regenerative capacity between early and established RA. C2C/CPII was universally improved and shifted toward CII regeneration in early RA, but not in established RA, regardless of responsiveness to infliximab. Restoration of C2C/CPII balance and the resulting cartilage regeneration is likely to be relevant to the degree to which the cartilage matrix has been damaged before starting anti-TNF therapy, rather than the magnitude of the suppression of systemic inflammation during anti-TNF therapy. A previous experimental study showed that mice with antigen- or zymosan-induced arthritis displayed reversible cartilage damage only when levels of collagen degradation were low [28]. This finding was corroborated by a human study using cartilage explants culture in vitro, in which aggrecanase-mediated aggrecan degradation did not influence the regenerative capacity of cartilage, but was markedly impaired after MMP-mediated aggrecan and collagen type II degeneration were initiated [29]. MMP-mediated aggrecan and collagen type II degeneration might thus represent a turning point for the reversibility of cartilage degradation. Therefore, whether RA is in the early or established phase (i.e., disease duration) does not appear critical.

In conclusion, ΔC2C/CPII offers a useful marker reflecting ongoing cartilage damage, which appears dissociated from inflammatory indices. As most measures of RA disease activity generally correlate with CRP, C2C/CPII appears to be of great clinical value as a CRP-independent marker, particularly when ongoing structural joint damage is evaluated during biological therapy in RA. The temporal course of C2C/CPII level during anti-TNF therapy indicated that CII turnover shifted toward CII synthesis in early RA, but not in established RA, potentially due to irreversible cartilage damage. The clinical significance of C2C/CPII should be further investigated in large-scale prospective studies to evaluate the feasibility of using this ratio as a surrogate marker for monitoring ongoing structural joint damage during the course of anti-rheumatoid therapy.

Supporting Information

Figure S1 Temporal course of the serum levels of various cytokines in patients with established RA during 54-week infliximab therapy. The data were measured using a Luminex® multiplex beads cytokine assay. Values were expressed as a proportion of each baseline value. Of note is the finding that serum levels of most inflammatory cytokines, including IL-6, TNF, and IL-17, were decreasing with decreasing CRP level over 54-week of infliximab therapy, whereas C2C/CPII level deteriorated over time.
(TIF)

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Author Contributions

Conceived and designed the experiments: YN TT. Performed the experiments: MN HN TK. Analyzed the data: YN TT. Contributed reagents/materials/analysis tools: HY YT. Wrote the paper: YN TM. Proof check of English: YT TM.

References

1. Emery P, Breedveld FC, Hall S, Durez P, Chang DJ, et al. (2008) Comparison of methotrexate monotherapy with a combination of methotrexate and etanercept in active, early, moderate to severe rheumatoid arthritis (COMET): a randomised, double-blind, parallel treatment trial. Lancet 372: 375–382.

2. Landewé R, van der Heijde D, Klareskog L, van Vollenhoven R, Fatenejad S (2006) Disconnect between inflammation and joint destruction after treatment with etanercept plus methotrexate: results from the trial of etanercept and methotrexate with radiographic and patient outcomes. Arthritis Rheum 54: 3119–3125.

3. Brown AK, Conaghan PG, Karim Z, Quinn MA, Ikeda K, et al. (2008) An explanation for the apparent dissociation between clinical remission and continued structural deterioration in rheumatoid arthritis. Arthritis Rheum 58: 2958–2967.
4. Garnero P, Piperno M, Gineyts E, Christgau S, Delmas PD, et al. (2001) Cross sectional evaluation of biochemical markers of bone, cartilage, and synovial tissue metabolism in patients with knee osteoarthritis: relations with disease activity and joint damage. Ann Rheum Dis 60: 619–626.
5. Lamers RJ, van Nesselrooij JH, Kraus VB, Jordan JM, Renner JB, et al. (2005) Identification of an urinary metabolite profile associated with osteoarthritis. Osteoarthritis Cartilage 13: 762–768.
6. Vignon E, Garnero P, Delmas P, Avouac B, Bettica P, et al. (2001) Respect of Ethics and Excellence in Science (GREES): Osteoarthritis SectionRecommendations for the registration of drugs used in the treatment of osteoarthritis: an update on biochemical markers. Osteoarthritis Cartilage 9: 289–293.
7. Poole AR, Alini M, Hollander AH. Cellular biology of cartilage degradation. In: Henderson B, Edwards JCW, Pettipher ER, eds. Mechanisms and models in rheumatoid arthritis London Academic Press; 1995, 163–204.
8. Poole AR. Cartilage in health and disease. In: McCarty OJ, Koopman WJ, eds. Arthritis and allied conditions. 12th ed Philadelphia: Lea and Febiger; 1993, 279–333.
9. Kong SY, Stabler TV, Criscione LG, Elliott AL, Jordan JM, et al. (2006) Diurnal variation of serum and urine biomarkers in patients with radiographic knee osteoarthritis. Arthritis Rheum 54: 2496–2504.
10. Mullan RH, Matthews C, Bresnihan B, FitzGerald O, King L, et al. (2007) Early changes in serum type II collagen biomarkers predict radiographic progression at one year in inflammatory arthritis patients after biologic therapy. Arthritis Rheum 56: 2919–2928.
11. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, et al. (1988) The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 31: 315–324.
12. Inoue E, Yamanaka H, Hara M, Tomatsu T, Kamatani N (2007) Comparison of Disease Activity Score (DAS)28- erythrocyte sedimentation rate and DAS28-C-reactive protein threshold values. Ann Rheum Dis 66: 407–409.
13. Cahue S, Sharma L, Dunlop D, Ionescu M, Song J, et al. (2007) The ratio of type II collagen breakdown to synthesis and its relationship with the progression of knee osteoarthritis. Osteoarthritis Cartilage 15: 819–823.
14. Kim TH, Stone M, Payne U, Zhang X, Ionescu M, et al. (2005) Cartilage biomarkers in ankylosing spondylitis: relationship to clinical variables and treatment response. Arthritis Rheum 52: 885–891.
15. Tomatsu S, Okamura K, Maeda H, Taketani T, Castrillon SV, et al. (2005) Keratan sulphate levels in mucopolysaccharidoses and mucolipidoses. J Inherit Metab Dis 28: 187–202.
16. Wakitani S, Nawata M, Kawaguchi A, Okabe T, Takaoka K, et al. (2007) Serum keratan sulfate is a promising marker of early articular cartilage breakdown. Rheumatology (Oxford) 46: 1652–1656.
17. Takeuchi T, Yamanaka H, Inoue E, Nagasawa H, Nawata M, et al. (2008) Retrospective clinical study on the notable efficacy and related factors of infliximab therapy in a rheumatoid arthritis management group in Japan: one-year outcome of joint destruction (RECONFIRM-2J). Mod Rheumatol 18: 447–454.
18. van den Broek M, Klarenbeek NB, Dirven L, van Schaardenburg D, Hulsmans HMJ, et al. (2011) Discontinuation of infliximab and potential predictors of persistent low disease activity in patients with early rheumatoid arthritis and disease activity score-steered therapy: subanalysis of the BeSt study. Ann Rheum Dis 70: 1389–1394.
19. Roos H, Dahlberg L, Hoerrner LA, Lark MW, Thonar EJ, et al. (1995) Markers of cartilage matrix metabolism in human joint fluid and serum: the effect of exercise. Osteoarthritis Cartilage 3: 7–14.
20. Thonar EJ, Glant T (1992) Serum keratan sulfate–a marker of predisposition to polyarticular osteoarthritis. Clin Biochem 25: 175–180.
21. Gordon CD, Stabler TV, Kraus VB (2008) Variation in osteoarthritis biomarkers from activity not food consumption. Clin Chim Acta 398: 21–6.
22. Rizkalla G, Reiner A, Bogoch E, Poole AR (1992) Studies of the articular cartilage proteoglycan aggrecan in health and osteoarthritis. Evidence for molecular heterogeneity and extensive molecular changes in disease. J Clin Invest 90: 2268–2277.
23. Månsson B, Carey D, Alini M, Ionescu M, Rosenberg LC, et al. (1995) Cartilage and bone metabolism in rheumatoid arthritis. Differences between rapid and slow progression of disease identified by serum markers of cartilage metabolism. J Clin Invest 95: 1071–1077.
24. Crnkic M, Månsson B, Larsson L, Geborek P, Heinegård D, Saxne T (2003) Serum cartilage oligomeric matrix protein (COMP) decreases in rheumatoid arthritis patients treated with infliximab or etanercept. Arthritis Res Ther 5: R181–185.
25. Young-Min S, Cawston T, Marshall N, Coady D, Christgau S, et al. (2007) Biomarkers predict radiographic progression in early rheumatoid arthritis and perform well compared with traditional markers. Arthritis Rheum 56: 3236–3247.
26. Morozzi G, Fabbroni M, Bellisai F, Cucini S, Simpatico A (2007) Low serum level of COMP, a cartilage turnover marker, predicts rapid and high ACR70 response to adalimumab therapy in rheumatoid arthritis. Clin Rheumatol 26: 1335–1338.
27. Mündermann A, Dyrby CO, Andriacchi TP, King KB (2005) Serum concentration of cartilage oligomeric matrix protein (COMP) is sensitive to physiological cyclic loading in healthy adults. Osteoarthritis Cartilage 13: 34–38.
28. van Meurs JB, van Lent PL, Holthuysen AE, Singer II, Bayne EK, Berg WB Van den (1999) Kinetics of aggrecanase- and metalloproteinase-induced neoepitopes in various stages of cartilage destruction in murine arthritis. Arthritis Rheum 42: 1128–1139.
29. Karsdal MA, Madsen SH, Christiansen C, Henriksen K, Fosang AJ, Sondergaard BC (2008) Cartilage degradation is fully reversible in the presence of aggrecanase but not matrix metalloproteinase activity. Arthritis Res Ther 10: R63.

連載

リウマチ性疾患
知っておきたいバイオマーカー 第1回

関節リウマチに対する生物学的製剤治療における血清軟骨バイオマーカーの重要性

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● Summary

関節リウマチ（RA）に対する治療は腫瘍壊死因子（TNF）阻害薬の登場により，症状の緩和のみならず，従来では到達困難とされてきた関節破壊の進行を抑制することが可能となった．しかし，骨破壊は抑制できても関節軟骨の破壊を抑制できるか否かについては不明である．われわれはTNF阻害薬を54週間投与し，その後の血清軟骨マーカーの変化を経時的に評価した．血清ヒアルロン酸（HA）値は早期RA，進行期RAともにCRPの減少に伴って低下したが，Ⅱ型コラーゲン代謝の指標であるC2C/CPⅡは早期RAでのみ低下した．Cartilage oligomeric matrix protein（COMP）およびケラタン硫酸（KS）では進行期RAでそれぞれ増加，減少した．興味深いことにC2C/CPⅡは早期RAではEULAR改善基準やCRPの変化に関係なく一貫して改善する傾向がみられたが，進行期RAでは一貫して悪化した．炎症と軟骨破壊とは必ずしも相関しないという過去の知見を考慮すると，臨床的評価基準のほかに，炎症とは独立した関節破壊進行を評価できる軟骨マーカーの存在意義は大きいと考えられる．

● Key Words

関節リウマチ（RA），関節軟骨，バイオマーカー，腫瘍壊死因子（TNF），関節破壊

はじめに

関節リウマチ（rheumatoid arthritis：RA）に対する治療は腫瘍壊死因子（tumor necrosis factor：TNF）阻害薬の登場により，症状の緩和のみならず，従来では到達困難とされてきた関節破壊の進行を抑制することが可能となった．とくに発症6ヵ月以内の早期RAにおいてはすでに臨床的寛解が治療の到達目標となっている．実際，早期RAでは，total sharp score（TSS）が負の値を示すような関節裂隙開大例も報告されており[1)2)]，関節破壊の抑制のみならず，関節軟骨の修復も期待される．しかし，従来の二次元のX線評価では軟骨びらんを正確に把握することはむずかしく，最近ではMRIのdGEMRIC，T2マッピング，T1rhoなどの新規撮像法の有効性が報告されている．一方，血清軟骨マーカーは以前からおもに変形性関節症（osteoarthritis：OA）の早期診断や治療効果判定マーカーとして注目されてきたが，日内変動や患者の活動性の影響を受けやすく臨床応用には至っていない[3)4)]．RAの抗TNF阻害療法の開始前後では軟骨代謝が大きく変化するため，日内変動や活動性の影響をうわまわる変化を血清軟骨マーカーで検出できる可能性がある．われわれは，インフリキシマブ（IFX）投与前後の血清サイトカインおよび軟骨マーカーの経時的変化を，早期RAと進行期RAに分けて検討をおこなったのでその結果を紹介する[5)]．

1．関節破壊進行の指標としての血清軟骨マーカー

関節軟骨に特異的に存在し，関節機能上重要な役割を担っている分子が理想的な軟骨マーカーといえる．詳細については本稿では割愛させていただくが，軟骨の主要マトリックスであるⅡ型コラーゲンやアグリカンの代謝産物は代表的な軟骨マーカーである．また，cartilage oligomeric matrix protein（COMP）などの軟骨基質の安

表❶ IFX 投与後 1 年間の RA 疾患活動性の経時的変化

	投与前	14 週	22 週	54 週
早期 RA（30 例）				
CRP [mg/dl]	4.12†	1.43**	1.02**	0.45**
DAS28-CRP	5.16	3.13**	2.74**	2.2**
MMP-3 [ng/ml]	342	167	116*	105*
ΔTSS（平均/中央値）				3.7/0
ΔTSS≦0 の割合 [%（例）]				73（19）
EULAR 改善基準 [%（例）]				
Good				63（19）
Moderate				27（8）
No response				10（3）
進行期 RA（27 例）				
CRP [mg/dl]	2.91	0.68**	0.66*	0.66*
DAS28-CRP	5.11	2.96**	2.76**	2.80**
MMP-3 [ng/ml]	298	92	98*	91*
ΔTSS（平均/中央値）				4.0/0
ΔTSS≦0 の割合 [%（例）]				70（16）
EULAR 改善基準 [%（例）]				
Good				41（11）
Moderate				37（10）
No response				22（6）

†数値はすべて平均値を示す．*p<0.05 v.s. 投与前（ベースライン）値，**p<0.001 v.s. 投与前（ベースライン）値

（Niki Y *et al*, 2012[5] より引用）

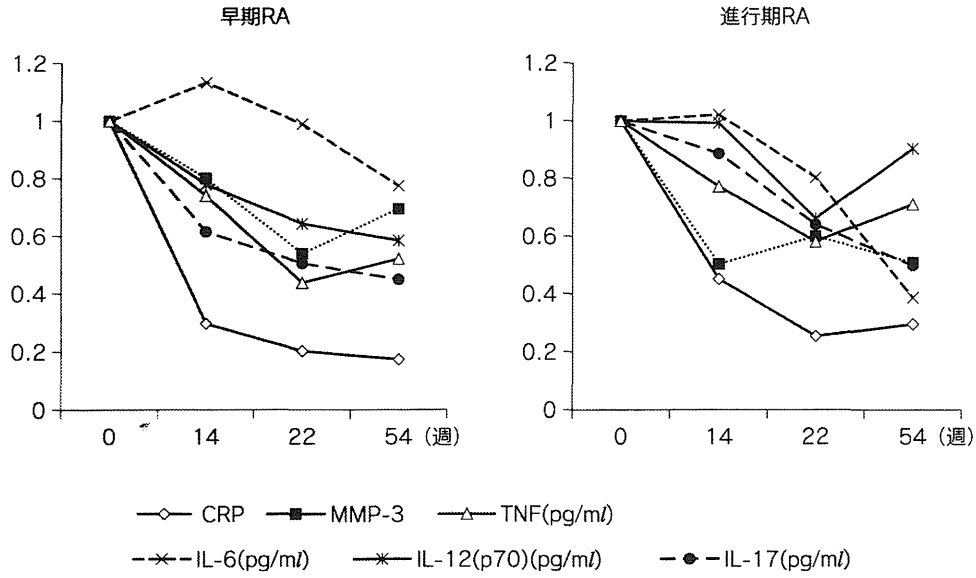
定化に関与する非コラーゲン性の蛋白も候補となる．一方，Ⅲ型コラーゲン，ヒアルロン酸（hyaluronic acid：HA），matrix metalloproteinases（MMPs）などは滑膜細胞が主要な産生源であり，滑膜炎マーカーとして受け入れられている．

2．早期および進行期 RA の IFX の臨床評価

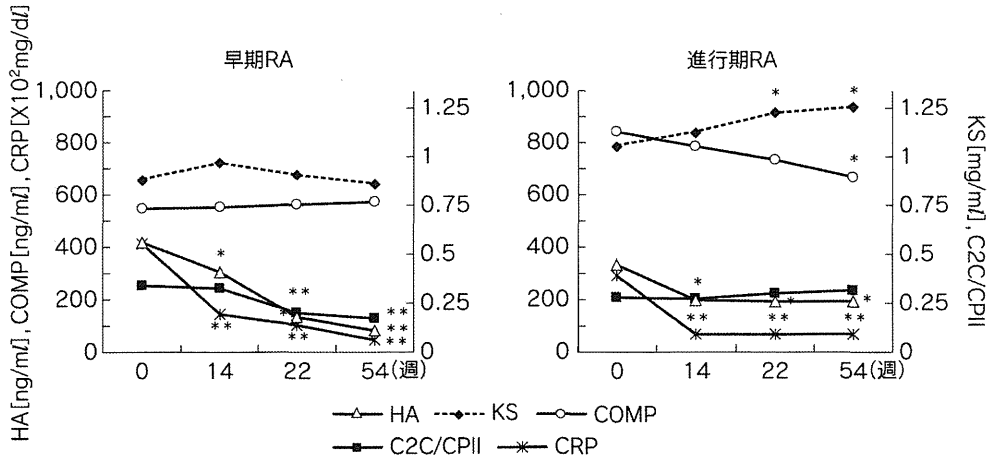
罹病期間 6 ヵ月以内の早期 RA 33 例（平均年齢 46 歳，DAS28-CRP 5.24），10 年以上の進行期 RA 33 例（平均年齢 56 歳，DAS28-CRP 4.80）を対象に IFX を 3 mg/kg，8 週間隔で投与し，54 週間の調査をおこなった．投与前，投与後 14 週，22 週，54 週間の 4 ポイントで血清を採取し，軟骨マーカーおよびサイトカイン濃度（TNF，IL-6，IL-12，IL-17）を測定した．同時に，RA の疾患活動性指標（DAS28-CRP），血液検査所見，X 線進行度（TSS），欧州リウマチ学会（EULAR）改善基準を記録した（表❶）．両群ともに 14 週以降，CRP および DAS28-CRP は有意に改善し，TSS の年間進行度の平均値（中央値）は，早期 RA で 3.7（0），進行期 RA で 4.0（0）となった．また，年間進行度が 0 以下を示す確率は，早期群 73%，進行期群 70%であり，これまでの国内のコホート研究と同等の臨床成績が得られた[6]．

3．血清サイトカイン濃度の経時的変化

IFX 投与後の血清サイトカイン濃度の経時的変化を早期 RA と進行期 RA に分けて検討した（図❶）．両群とも IFX と結合していないフリー TNF は 14 週，22 週と順調に低下しているが，54 週で軽度上昇に転じている．これに連動するように早期 RA では MMP-3，進行期 RA では IL-12 が若干反転上昇に転じている．一方，IL-17，CRP は両群とも IFX 投与後，一貫して低下する傾向にあった．今回のコホートは IFX のトラフ値が低下した二次無効例を少なからず含んでいることから，フリー TNF の平均値は 54 週で若干高くなっている可能性が考えられる．血清 IL-6 は TNF に遅れて低下する傾向がみられたが，IL-6 と連関性の高い CRP は早期から著明に低下していた．



図❶ IFX 投与後の血清サイトカインの経時的変化
投与前を 1 として表示している.

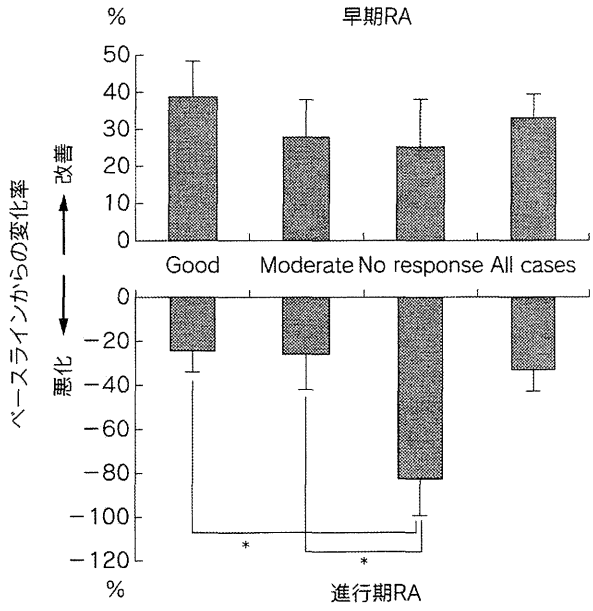


図❷ IFX 投与後の血清軟骨マーカーの経時的変化
*p<0.05 v.s. 投与前（ベースライン値），**p<0.001 v.s. 投与前（ベースライン値）
（Niki Y *et al*, 2012[5] より引用）

4. 血清軟骨マーカーの経時的変化

軟骨マーカーの経時的変化についても早期 RA と進行期 RA に分けて検討した．もし，IFX に関節軟骨破壊を抑制する効果があれば，血清軟骨マーカーは低下するはずである．血清 HA，COMP，ケラタン硫酸（keratan sulfate：KS），Ⅱ型コラーゲン分解/合成比（type Ⅱ collagen（CⅡ）-related neoepitope/type Ⅱ procollagen carboxy-propeptide：C2C/CPⅡ）の測定結果を図❷に示す．

早期 RA においては血清 HA と C2C/CPⅡは CRP と連動して低下したが，COMP と KS には有意な変化はみられなかった．一方，進行期 RA では COMP と KS のベースライン値が早期 RA よりも高く，COMP は漸減傾向，KS は漸増傾向を示した．また HA は早期 RA と同様に CRP とともに経時的に低下したが，C2C/CPⅡの低下はみられなかった．これらの結果から，C2C/CPⅡすなわちⅡ型コラーゲン代謝は，早期 RA でのみ合成系にシフトし，進行期 RA では改善しないことが明らかとなった．一方，



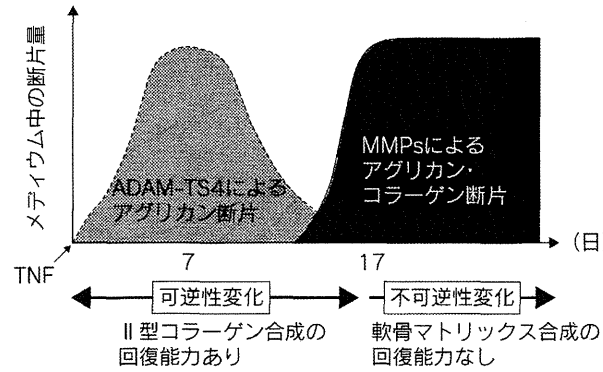
図❸ Ⅱ型コラーゲン代謝マーカー（C2C/CPII）の54週後の変化
*$p<0.05$

（Niki Y *et al*, 2012[5] より引用）

COMPに関してはベースライン値が高かった進行期RAでは低下したが，ベースライン値の低い早期RAでは変化しなかった．また，KSは以前から軟骨破壊マーカーとして考えられてきたが，われわれの結果ではアグリカン合成を反映しており，進行期RAでは炎症がコントロールされた結果，増加したと考えられた．過去のOAに関する研究でも軟骨変性が軽い患者ほどKS値が高いと報告されており，KSの増加は軟骨合成を示唆していると考えられる[7]．

5. EULAR改善基準とⅡ型コラーゲン代謝

EULAR改善基準にしたがってIFXの治療効果を3段階に分類し，Ⅱ型コラーゲン分解/合成比C2C/CPⅡの改善度を比較した（図❸）．コホート全体で検討すると，good，moderate，no responseの順に改善度は小さくなり，C2C/CPⅡの改善度はリウマチの疾患活動性改善度と相関していた．興味深いことに早期RAと進行期RAに分けて検討すると，早期RAではno responseであってもC2C/CPⅡは一貫して改善傾向を示し，反対に進行期RAではgood responseであっても一貫して悪化傾向を示すことが明らかとなった．



図❹ 関節軟骨器官培養におけるアグリカンおよびコラーゲン断片の出現と軟骨破壊の不可逆性

（Karsdal MA *et al*, 2008[10] を参考に作成）

6. 関節軟骨破壊の不可逆性変化

過去のRA動物モデルの研究やヒトRAの臨床データから軟骨破壊と炎症は必ずしも相関しないと考えられている[2)8)9)]．実際，われわれのデータでもⅡ型コラーゲン代謝の指標であるC2C/CPⅡは，早期RAであればCRPやMMP-3が低下しないnonresponderでも改善がみられている（図❸）．また，進行期RAにおいてCRPが著明に改善したgood responderであっても，C2C/CPⅡが改善しなかったことは納得できる結果である．ここで考察しなければならないのは，関節軟骨破壊の不可逆性についてである．われわれの結果は，早期RAでのみⅡ型コラーゲン代謝は改善することを示したが，早期RAと進行期RAの軟骨の質的な違いはどこにあるのかについて追及すべきである．この点について研究したKarsdalら[10]の興味深い実験結果がある．彼らはヒト関節軟骨の器官培養系においてTNFを添加すると軟骨マトリックスの破壊が起こるが，17日目でその破壊は不可逆性になると論じた．この実験ではインスリン様増殖因子（insulin-like growth factor：IGF）-1添加によるⅡ型コラーゲン合成能力で不可逆性を評価しているが，TNF添加後17日目以降はⅡ型コラーゲン合成がIGF-1を添加しても回復しなくなることを明らかにした．A disintegrin and metalloproteinase with thrombospondin motifs（ADAM-TS）4によるアグリカンの破壊は早期からみられるが，17日目

以降はMMPによるアグリカンやコラーゲンの破壊が主となり，IGF-1抵抗性になると考えられる（図❹）．この結果は，MMPによる軟骨マトリックス破壊がスタートする前にRAの病勢をコントロールすることが，軟骨破壊抑制の鍵であることを示唆している．実地臨床において，本当にこのような理論が成立するか否かについては今後の研究課題である．

おわりに

生物学的製剤の登場が何をもたらしたかを考えた場合，その第一番目は関節破壊の進行抑制であることに異論はない．しかし，関節破壊進行の正確な評価は，DAS28や最近のBoolean，SDAI，CDAIをはじめとする臨床的寛解基準を用いたとしても困難である．炎症とは乖離した関節軟骨の破壊を評価できる血清軟骨マーカーは実地臨床において有用である．しかし，現行の血清軟骨マーカーは，日内変動が存在する点[11]や年齢，活動性の影響を受けやすい点[12)13)]で絶対的指標としては使用できない．これらは今後の解決すべき課題である．

文 献

1) Emery P *et al*：Comparison of methotrexate monotherapy with a combination of methotrexate and etanercept in active, early, moderate to severe rheumatoid arthritis（COMET）：a randomised, double-blind, parallel treatment trial. *Lancet* **372**：375-382, 2008
2) Landewé R *et al*：Disconnect between inflammation and joint destruction after treatment with etanercept plus methotrexate：results from the trial of etanercept and methotrexate with radiographic and patient outcomes. *Arthritis Rheum* **54**：3119-3125, 2006
3) Garnero P *et al*：Cross sectional evaluation of biochemical markers of bone, cartilage, and synovial tissue metabolism in patients with knee osteoarthritis：relations with disease activity and joint damage. *Ann Rheum Dis* **60**：619-626, 2001
4) 伊達秀樹ほか：血清マーカーによる変形性関節症の病態評価，進行予知．別冊整形外科 **53**：60-66，2008
5) Niki Y *et al*：Clinical significance of cartilage biomarkers for monitoring structural joint damage in rheumatoid arthritis patients treated with anti-TNF therapy. *PLoS One* **7**：e37447, 2012
6) Takeuchi T *et al*：Retrospective clinical study on the notable efficacy and related factors of infliximab therapy in a rheumatoid arthritis management group in Japan：one-year outcome of joint destruction（RECONFIRM-2J）. *Mod Rheumatol* **18**：447-454, 2008
7) Wakitani S *et al*：Serum keratan sulfate is a promising marker of early articular cartilage breakdown. *Rheumatology*（*Oxford*）**46**：1652-1656, 2007
8) van den Berg WB：Joint inflammation and cartilage destruction may occur uncoupled. *Springer Semin Immunopathol* **20**：149-164, 1998
9) Brown AK *et al*：An explanation for the apparent dissociation between clinical remission and continued structural deterioration in rheumatoid arthritis. *Arthritis Rheum* **58**：2958-2967, 2008
10) Karsdal MA *et al*：Cartilage degradation is fully reversible in the presence of aggrecanase but not matrix metalloproteinase activity. *Arthritis Res Ther* **10**：R63, 2008
11) Kong SY *et al*：Diurnal variation of serum and urine biomarkers in patients with radiographic knee osteoarthritis. *Arthritis Rheum* **54**：2496-2504, 2006
12) Roos H *et al*：Markers of cartilage matrix metabolism in human joint fluid and serum：the effect of exercise. *Osteoarthritis Cartilage* **3**：7-14, 1995
13) Gordon CD *et al*：Variation in osteoarthritis biomarkers from activity not food consumption. *Clin Chim Acta* **398**：21-26, 2008

2．関節リウマチにおける骨・軟骨破壊のバイオマーカー

2）生物学的製剤時代における血清軟骨マーカーの臨床的意義について

二木 康夫* 竹内 勤**

生物学的製剤の登場によって関節リウマチ（RA）の治療は格段に進歩し，多くの患者が臨床的寛解基準を満たすようになった。しかし，関節破壊が進行していく症例が少なからず存在するのも事実である。われわれはTNF阻害薬を54週間投与し，その関節破壊抑制効果を血清軟骨マーカーを用いて評価した。血清ヒアルロン酸値は早期RA，進行期RAともにCRPの減少に伴って低下したが，II型コラーゲン代謝の指標であるC2C/CPIIは早期RAでのみ低下した。COMPおよびケラタン硫酸では，進行期RAでそれぞれ減少，増加した。興味深いことに，C2C/CPIIは早期RAではEULAR改善基準やCRPの変化に関係なく一貫して改善する傾向が見られたが，進行期RAでは一貫して悪化した。炎症と軟骨破壊とは必ずしも相関しないという過去の知見を考慮すると臨床的評価基準の他に，炎症とは独立した関節破壊進行を評価できる軟骨マーカーの存在意義は大きいと考えられる。

Bone and cartilage destruction in RA and its intervention.

Clinical significance of serum cartilage biomarkers in the treatment of rheumatoid arthritis with biological therapy.

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With the current use of biologics in RA, numerous patients can achieve clinical remission, however structural joint damage occurs in substantial numbers of the patients. The present study assessed cartilage damage during 54-week anti-TNF therapy, using serum cartilage markers, and potential advantages of these markers were evaluated. Levels of serum hyaluronan decreased with decreasing levels of CRP in both early and established RA, whereas indicator of type II collagen synthesis/degradation, C2C/CPII decreased only in early

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RA group. Levels of COMP and keratin sulfate significantly decreased and increased compared to baseline, respectively, by week 54 in established RA. Strikingly, C2C/CP II levels were universally improved in early RA, regardless of CRP levels or EULAR response grade. In contrast, C2C/CP II levels universally worsened in established RA, even though patients achieved good response. As a role of surrogate marker reflecting therapeutic efficacy of biological therapy, C2C/CP II appears particularly useful for determining the degree of ongoing structural joint deterioration, which is dissociated from clinical assessment of disease activity in RA.

はじめに

関節リウマチ（RA）の治療は生物学的製剤の登場により革命的なパラダイムシフトがもたらされ，症状の緩和のみならず，従来では到達困難とされた関節破壊進行の抑制までもが可能になった。生物学的製剤の治療経験が蓄積されてきた現在，臨床的寛解，画像的寛解，そしてドラッグフリー寛解という高い目標設定がなされている。特に早期RAでは，シャープスコアが負の値を示すような関節裂隙開大例も報告されており[1) 2)]，関節破壊の抑制のみならず，関節軟骨の修復も期待される。しかし，二次元のX線写真によって関節軟骨の状態を正確に評価することは難しく，最近では高感度MRIや超音波を用いて評価する試みがなされている。

血清軟骨マーカーは，以前から主に変形性関節症（OA）において早期診断や治療効果判定指標として注目されてきたが，日内変動や患者の活動性の影響を受けやすく日常診療に普及するレベルには至っていない[3)~6)]。一方，RAにおける抗腫瘍壊死因子（TNF）抗体療法の開始前後ではOAに比し，軟骨代謝が大きく変化する可能性があり，血清軟骨マーカーの有効性が期待される。われわれは，インフリキシマブ（レミケード®）投与前後の血清軟骨マーカーの経時的変化を早期RAと進行期RAに分けて検討したので，本稿ではその結果を紹介し，軟骨マーカーの臨床的意義について考察する。

関節破壊進行の指標としての関節マーカー

生物学的製剤時代の軟骨マーカーの意義について述べる前に，関節マーカーの分類について触れておきたい。関節内に特異的に存在し，関節機能上重要な役割を担っている分子が理想的な関節マーカーといえる。関節マーカーは，軟骨マーカーと滑膜炎マーカーの大きく2つに分類される。

現在，RAまたはOAにおいて関節破壊進行の指標として使用されているものを**表1**に示した。軟骨の主要マトリックスであるII型コラーゲンやアグリカンの代謝産物は，代表的な軟骨マーカーである。また，cartilage oligomeric matrix protein（COMP）などの軟骨基質の安定化に関与する非コラーゲン性の蛋白も候補となる。一方，III型コラーゲン，ヒアルロン酸（hyaluronan：HA），matrix metalloproteinases（MMPs）などは，滑膜細胞が主要な産生源であることがわかっており，軟骨マーカーというよりは，滑膜炎マーカーとして認められている。

RA：rheumatoid arthritis（関節リウマチ），OA：osteoarthritis（変形性関節症）
TNF：tumor necrosis factor（腫瘍壊死因子）
COMP：cartilage oligomeric matrix protein（軟骨オリゴマー基質タンパク質），HA：hyaluronan（ヒアルロン酸）
MMPs：matrix metalloproteinases（マトリックスメタロプロテアーゼ）

表1 関節マーカーの分類

関節マーカーとして，軟骨の主要マトリックスであるII型コラーゲンやアグリカンの代謝産物，非コラーゲン性の蛋白などが候補となる。滑膜細胞が主要な産生源であるIII型コラーゲン，ヒアルロン酸，MMPsなどは，滑膜炎マーカーとして認められている。

軟骨マーカー
●破壊マーカー
II型コラーゲン関連
・C2C (type II collagen〔CII〕-related neoepitope)
・C12C(carboxy-terminus of three-quarter peptide from cleavage of CI and CII)
・CTX-II (C-terminal crosslinking telopeptide of type II collagen)
アグリカン関連
・ケラタン硫酸 (epitope：5D4，ANP9)
・コンドロイチン硫酸 (C6S)
非コラーゲン蛋白
・COMP (cartilage oligomeric matrix protein)
●合成マーカー
II型コラーゲン関連
・CPII (type II procollagen carboxy-propeptide)
アグリカン関連
・コンドロイチン硫酸 (epitope：CS-846，3B3，7D4)
非コラーゲン蛋白
・YKL-40 (human cartilage glycoprotein-39)
・CD-RAP (cartilage-derived retinoic acid sensitive protein)
滑膜炎マーカー
III型コラーゲン関連
・PIIINP (procollagen III N-terminal propeptide)
・Glc-Gal-PYD
非コラーゲン性蛋白
・ヒアルロン酸
・YKL-40
蛋白分解酵素
・MMPs (matrix metalloproteinases)

(筆者作成)

早期および進行期RAのインフリキシマブの臨床評価

罹病期間6カ月以内の早期RA 33例(平均年齢46歳，DAS28-CRP 5.24)，10年以上の進行期RA33例(平均年齢56歳，DAS28-CRP 4.80)を対象にインフリキシマブ（レミケード®）を3mg/kg，8週間隔で投与し，54週間の調査を行った。投与前，投与後14週，22週，54週間の4ポイントで血清を採取し，軟骨マーカーを測定すると同時に，RAの疾患活動性指標(**DAS28-CRP**)，血液検査所見，X線進行度(シャープスコア)，欧州リウマチ学会(**EULAR**)改善基準を記録した。両群ともに14週以降，CRPおよびDAS28-CRPは有意に改善し，シャープスコアの年間進

DAS28-CRP：Disease Activity Score 28-C-reactive protein (RAの疾患活動性指標)
EULAR：European League Against Rheumatism (欧州リウマチ学会)

行度の平均値（中央値）は，早期RAで3.7（0），進行期RAで4.0（0）となった。また，年間進行度が0以下を示す確率は，早期群73%，進行期群70%であった。EULAR改善基準別では，早期RAでgood 63%，moderate 27%，no response 10%，進行期RAでgood 41%，moderate 37%，no response 22%であった。これらの結果から，われわれのコホートにおいても他のコホート研究と同等の臨床成績が得られたと判断できた[7]。

血清軟骨マーカーの経時的変化

レミケード® に対し良好な治療効果を示した早期RAと進行期RAのコホートにおいて，軟骨マーカーの経時的変化を検討した。なお，投与時反応または二次無効のため投与を中止した早期RAの3例，進行期RAの6例を除外した。構造的寛解（シャープスコア年間進行度＜0.5）を達成するには，骨代謝の改善（≒骨びらん）のみならず，軟骨代謝の改善（≒関節裂隙の狭小化）の両者が必要である。関節裂隙狭小化の進行が抑えられた症例では，各血清軟骨マーカーは低値を示すことが予想される。

血清中のHA，COMP，ケラタン硫酸（KS），II型コラーゲン分解/合成比（type II collagen〔C

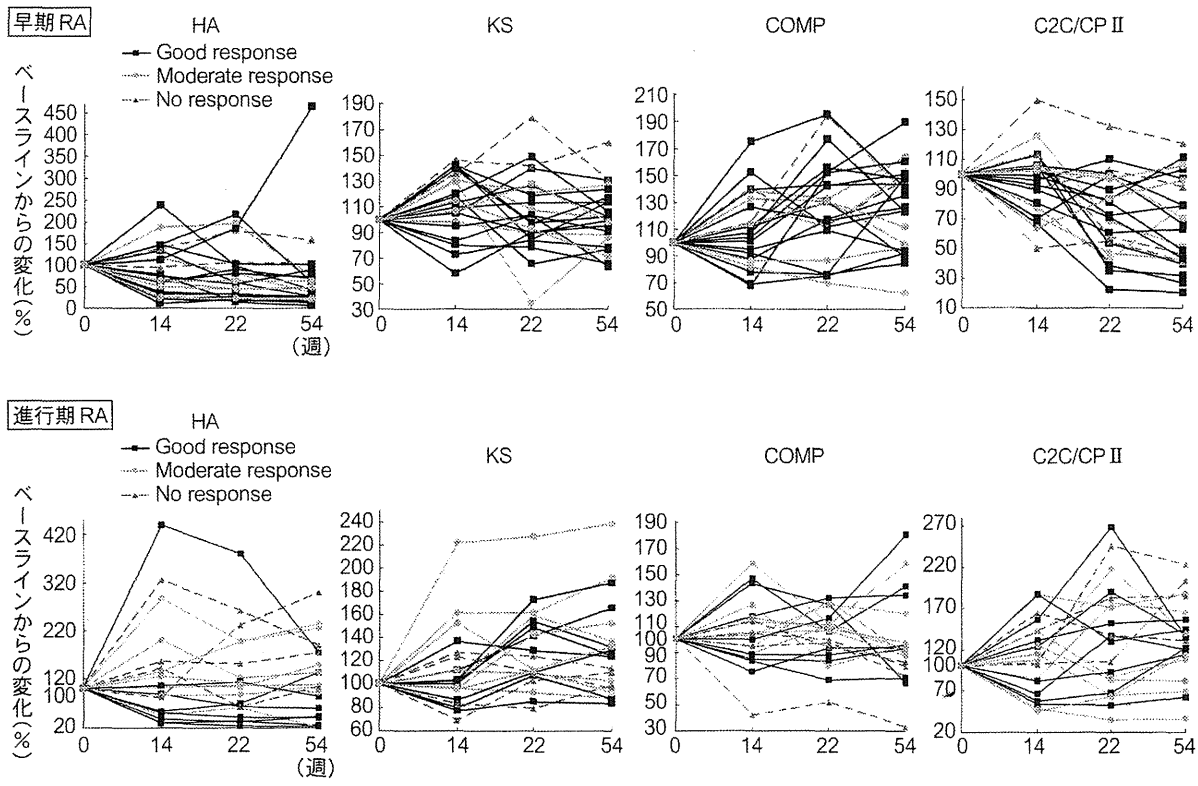

図1　各血清軟骨マーカーの経時的変化

ベースライン値を100として数値化し，EULAR改善基準に従って分類した。

早期RAにおいて血清HAとC2C/CP IIは経時的に低下する傾向を示したが，COMPとKSには有意な変化はみられなかった。一方，進行期RAではいずれのマーカーについても一定の傾向を認めなかった。

C2C/CP II：II型コラーゲン分解/合成比，COMP：cartilage oligomeric matrix protein，EULAR：欧州リウマチ学会，HA：ヒアルロン酸，KS：ケラタン硫酸

（筆者作成）

Ⅱ)-related neoepitope：**C2C**/type Ⅱ procollagen carboxy-propeptide：**CP**Ⅱ）について，早期および進行期RA患者個々の経時的変化を**図1**に示した。早期RAにおいて血清HAとC2C/CPⅡは経時的に低下する傾向を示したが，COMPとKSには有意な変化はみられなかった。一方，進行期RAではいずれのマーカーについても一定の傾向を認めなかった。個々のマーカーのベースライン値と54週後の値の平均値をグラフ化すると，早期RAにおいて血清HAとC2C/CPⅡはCRPと連動して低下し，統計学的に有意差が認められた(**図2a**)。進行期RAではCOMPとKSのベースライン値が早期RAよりも高く，COMPは漸減傾向，KSは漸増傾向を示した(**図2b**)。またHAは早期RAと同様にCRPとともに減少したが，C2C/CPⅡの減少はみられなかった。

本結果からC2C/CPⅡすなわち，Ⅱ型コラーゲン代謝は，早期RAでのみ合成系にシフトし，進行期RAでは改善しないことが明らかとなった。一方，COMPに関してはベースライン値が高かった進行期RAでは減少したが，ベースライン値の低い早期RAでは変化しなかった。また，KSは以前から軟骨破壊マーカーとして考えられてきたが(**表1**)，われわれの結果ではアグリカンの合成マーカーとしての性質を持ち，RAの病勢がコントロールされた結果，進行期RAにおいて増加したと考えられた。過去のOAに関する報告でも軟骨変性が軽い患者ほどKSが高いというデータ

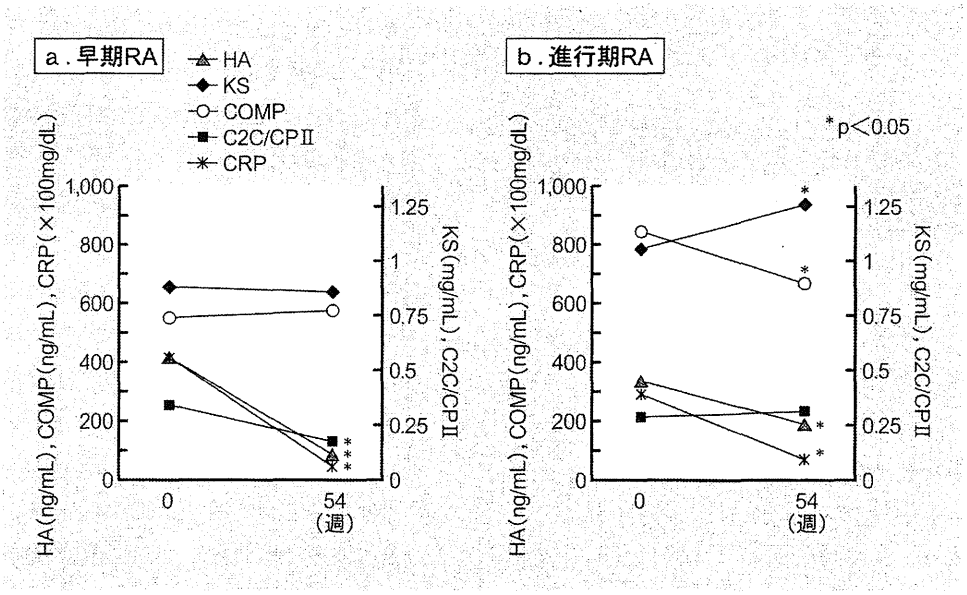

図2 各血清軟骨マーカーの54週後の変化（平均値）

早期RAでは，血清HAとC2C/CPⅡはCRPと連動して減少し，統計学的に有意差が認められた。進行期RAでは，COMPとKSのベースライン値が早期RAよりも高く，COMPは漸減傾向，KSは漸増傾向を示した。

C2C/CPⅡ：Ⅱ型コラーゲン分解/合成比，COMP：cartilage oligomeric matirix protein，CRP：C反応性蛋白，HA：ヒアルロン酸，KS：ケラタン硫酸

（筆者作成）

KS：keratan sulfate（ケラタン硫酸）

C2C/CPⅡ：type Ⅱ collagen（CⅡ）-related neoepitope/type Ⅱ procollagen carboxy-propeptide（Ⅱ型コラーゲン分解/合成比）

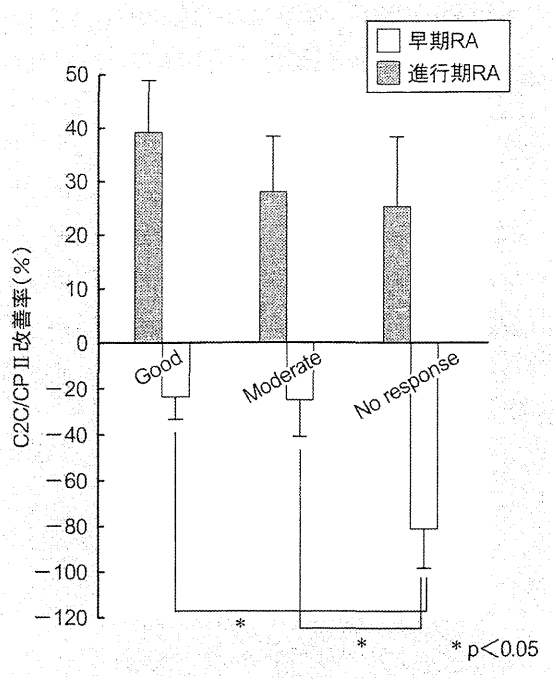

図3　EULAR改善基準別のC2C/CPⅡ改善率

早期RAでは，no responseであってもC2C/CPⅡは一貫して改善化傾向を示し，反対に進行期RAではgood responseであっても一貫して悪化傾向を示した。

C2C/CPⅡ：Ⅱ型コラーゲン分解/合成比，EULAR：欧州リウマチ学会

（筆者作成）

が得られており，われわれと同様にKSを合成マーカーと考えるグループもある[8]。

EULAR改善基準とⅡ型コラーゲン代謝

EULAR改善基準にしたがってインフリキシマブの治療効果を3段階に分類し，Ⅱ型コラーゲン分解/合成比（C2C/CPⅡ）の改善度を比較した（**図3**）。興味深いことに早期RAと進行期RAに分けて検討すると，早期RAではno responseであってもC2C/CPⅡは一貫して改善傾向を示し，反対に進行期RAではgood responseであっても一貫して悪化傾向を示すことが明らかとなった。

血清軟骨マーカーとRA疾患活動性指標との相関

今回，調査した軟骨マーカーの中でCRP，DAS28，MMP-3の疾患活動性指標と相関が認められたC2C/CPⅡとHAに関して，各々のベースライン値を引いた改善度（⊿C2C/CPⅡ，⊿HA）を計算し，投与後54週時における相関係数およびp値を求めた（**表2**）。⊿C2C/CPⅡは早期RAにおいてのみ，⊿HAは早期および進行期RAにおいて⊿CRPと有意な相関を認めた。さらに⊿C2C/CPⅡ，⊿HAはともに早期RAにおいて⊿DAS28と相関を認めた。過去のRA動物モデルの研究やヒトRAの臨床データから，軟骨破壊と炎症は必ずしも相関しないと考えられてきたため[2) 9) 10)]，CRPを交絡因子と考え偏相関係数を算出した。すると興味深いことに，⊿C2C/CPⅡはCRPの影響を除いても⊿DAS28と相関関係にあったが，⊿HAでは⊿DAS28との相関は消失し54週時のMMP-3にのみ相関が認められた。

この結果はCRPを炎症の最も鋭敏なマーカーと考えれば，⊿C2C/CPⅡと⊿DAS28の連動は炎症の消退すなわちCRP値の低下とは直接関係がないことを示している。進行期RAにおいてCRP値が低下しているにも関わらず，⊿C2C/CPⅡが改善しないのはこの結果に一致する。

実地臨床における血清軟骨マーカーの意義

実地臨床においてDAS臨床的寛解基準を満たしていても滑膜炎が残存し，関節破壊が経年的に進行する例が存在する。特にメトトレキサート（**MTX**）で寛解を達成した場合，関節破壊の進行に注意しなければならない。造影MRIやカラードップラーの画像診断を適宜取り入れながら，軟骨破壊を正確にモニターする方法が報告されているが，軟骨マーカーを関節破壊進行のサロゲート

MTX：methotrexate（メトトレキサート）

表2 各血清軟骨マーカーとRA疾患活動性指標の相関（54週時）

早期RAにおいて⊿C2C/⊿CPⅡ，⊿HAはともに⊿DAS28と相関していたが，⊿C2C/⊿CPⅡはCRPの影響を排除しても相関が認められた。

		相関係数（p値）	偏相関係数（p値）
早期RA			
⊿C2C/⊿CPⅡ	vs. ⊿CRP	0.50 (0.01)	———
	vs. ⊿DAS28	0.52 (0.01)	0.49 (0.02)
⊿HA	vs. ⊿CRP	0.45 (0.03)	———
	vs. ⊿DAS28	0.43 (0.04)	n.s.
進行期RA			
⊿C2C/⊿CPⅡ	vs. ⊿CRP	n.s.	———
	vs. ⊿DAS28	n.s.	n.s.
⊿HA	vs. ⊿CRP	0.43 (0.04)	———
	vs. ⊿DAS28	n.s.	n.s.

n.s.：有意差なし

C2C/CPⅡ：Ⅱ型コラーゲン分解/合成比，CRP：C型反応性蛋白，DAS28：Disease Activity Score 28，HA：ヒアルロン酸

（筆者作成）

マーカーとして利用できれば，血液検査ですむため利便性が高い。生物学的製剤を投与することにより，骨びらんは修復されるが，失われた関節軟骨が修復されるかについては不明であり，今後の検討課題である。

われわれのコホートにおいてシャープスコアの年間進行度が0以下を示す割合は，早期，進行期RAともに60%を超えているが，常にメカニカルストレスが加わる下肢荷重関節では長期的にみると軟骨破壊が進行する可能性がある。今回の結果から，C2C・CPⅡバランスに注目すると早期RAでは生物学的製剤の使用によって軟骨合成へシフトする可能性があるが，進行期RAでは変化しないことが明らかとなった。また，C2C/CPⅡはCRP，DAS28，MMP-3と直接的な相関関係はないことから，これらの疾患活動性指標とは独立した関節破壊進行のサロゲートマーカーとして期待される。

しかし，血清軟骨マーカーには少なからず，問題点も存在する。1つはベースライン値が患者ごとに異なるため，ベースラインからの改善度で評価しなければならず，絶対的指標にはなり得ないこと，2つ目は，日内変動があり，採血する時間を一定にしなければならないこと[11]，3つ目は，患者の年齢や活動性の影響を受けやすいこと[12)13)]，4つ目は高齢者の場合，投与前から存在する膝や脊椎のOA変化の影響を受けることなどである。後者2つは1番目に含まれる問題であり，今後RAの病勢以外の患者背景因子の影響を受けにくい絶対的指標となる軟骨マーカーの開発が期待される。

おわりに

生物学的製剤の登場が何をもたらしたかを考えた場合，その第一番目は関節破壊の進行抑制であることに異論はない。しかし，関節破壊進行の正確な評価は，DAS28や最近のBoolean，**SDAI**（Simple Disease Activity Index），**CDAI**（Clini-

SDAI：Simple Disease Activity Index，**CDAI**：Clinical Disease Activity Index