Increased remodelling of interstitial collagens and basement membrane is suppressed by treatment in patients with rheumatoid arthritis: serological evaluation of a one-year prospective study of 149 Japanese patients

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Abstract

Objective

This prospective study aimed to use serological biomarkers for evaluation of, connective tissue turnover, in a population of 149 Japanese patients with rheumatoid arthritis (RA). It was aimed to investigate how the connective tissue was affected by treatment at follow-up after 1 year (± 6 weeks) with either methotrexate (n=23) alone, or in combination with: adalimumab (n=49), tofacitinib (n=27) or tocilizumab (n=50).

Methods

Clinical characteristics were collected and connective tissue turnover, was evaluated by 4 serological biomarkers: C1M and C3M reflect degradation of types I and III collagen in interstitial tissue; C4M, reflecting degraded type IV collagen of the basement membranes; and CRPM, a marker of degraded C-reactive protein. Evaluated biomarker levels were measured at baseline and at follow-up. Levels were compared to the reference levels of healthy individuals.

Results

The four evaluated biomarkers were all elevated at baseline in patients with RA compared to healthy individuals. The biomarkers were higher in RA patients compared to healthy individuals at baseline and they were all significantly correlated with disease activity score of 28 joint (DAS28) ($p<0.0001$). The biomarker levels were all significantly decreased in all four patient groups at follow-up in all of the four treatment groups.

Conclusion

Rheumatoid joint inflammation elicits enhanced turnover of major collagen constituents of the synovial membrane and this abnormal pathway may be implicated in erosive progression. Evaluations of the applied biomarkers: C1M, C3M, C4M and CRPM, indicate that the pathologically enhanced tissue turnover was attenuated, by all of the four studied treatments.

Key words

rheumatoid arthritis, biomarkers, extra cellular matrix, DMARDs, collagens

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Competing interests: N.S. Gudmann, M.A. Karsdal, and A.-C. Bay-Jensen are full time employees at, and M.A. Karsdal holds stocks in, Nordic Bioscience. Nordic Bioscience is a privately-owned, small-medium sized enterprise (SME) partly focused the development of biomarkers for rheumatic and fibrotic diseases. Y. Tanaka has received consulting fees, speaking fees, and/or honoraria from Abbvie, Daiichi-Sankyo, Chugai, Takeda, Mitsubishi-Tanabe, Bristol-Myers, Astellas, Eisai, Janssen, Pfizer, Asahi-Kasei, Eli Lilly, GlaxoSmith-Kline, UCB, Teijin, MSD, Santen and has received research grants from Mitsubishi-Tanabe, Takeda, Chugai, Astellas, Eisai, Taisho-Toyama, Kyowa-Kirin, Abbvie, Bristol-Myers.
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Background

Rheumatoid arthritis (RA) is an autoimmune and inflammation-driven disease, which mainly affects peripheral synovial joints. Synovitis, progressive bone erosions and cartilage destruction are some of the characteristics of RA. In addition, patients with RA tend to have extra-articular manifestations such as endothelial dysfunction even at an early stage of disease.

With the growing armamentarium of RA therapies, that includes agents with quite different modes of action, it is possible to obtain clinical remission at least for some patients (1-3). Current treatments include those used in this study, namely methotrexate (MTX) alone or in combination with adalimumab (ADA), tofacitinib (TOFA) and tocilizumab (TCZ). MTX is a first-line treatment and one of the most effective treatments (4) which is often prescribed as soon as patients are diagnosed (5). ADA is an anti-tumour necrosis factor (TNF)-α agent, which was among the first biological disease-modifying anti-rheumatic drugs (DMARDs) approved for RA (6) and is still widely used even in the early phases of RA (7). TOFA is a small-molecule drug that targets the intracellular signalling molecules, Janus activating kinase (JAK). The inhibition of interleukin (IL)-6 signals by TCZ reduces systemic inflammation, RA synovitis, and bone and cartilage damage, and may have beneficial cardiovascular effects (8). Both TCZ and TOFA have recently been approved for moderately to severely active RA in patients who are intolerant of or respond inadequately to a synthetic DMARD alone (9-11). Much research has been conducted in order to determine how these agents lower disease activity and pain as well as delaying or even preventing further bone and cartilage damage. However, less research has focused on how the connective tissue is affected by RA pathogenesis and/or the treatment of choice.

In inflammatory conditions, including RA, epithelial cells in tissue are damaged, thus exposing the basement membrane to degradation and enabling the influx of inflammatory cells to the deeper interstitial tissue, which is changing remodelling rate as a consequence. One of the main constituents of the basement membrane is type IV collagen (12). Degradation of type IV collagen by matrix metalloproteinases (MMPs) can be quantified by the biomarker C4M (13). The main constituents of the interstitial tissue are type I and III collagen. Degradation of type I and III collagens can be quantified by serum levels of the MMP-degraded collagen C1M (14-16) and C3M (16-19), respectively. Following surface cell damage, the underlying tissues are destroyed by proteases, giving rise to the release of neo-epitopes as shown in Figure 1. The released neo-epitopes includes the measurable C1M, C3M and C4M, which can be quantified in the serum.

The aim of this prospective study was to investigate if remodelling of basement and interstitial collagens, in patients with RA, is attenuated after treatment with MTX alone, or TCZ+MTX, ADA+MTX or TOFA+MTX, each of which have different modes of actions. The biomarkers, C1M, C3M and C4M were selected to assess degradation of the interstitial tissue and basement membrane in addition to the inflammatory marker CRPM. Levels of C1M and C3M in RA patients have been shown to be elevated and modulated in response to therapy as well as being prognostic for disease progression (15, 20). The biomarker C4M reflects type IV collagen degradation (13) originating from the basement membrane. Increased levels of C4M have previously been detected in ankylosing spondylitis (18). CRPM is a biomarker reflecting MMP-dependent degradation of C-reactive protein (CRP). While CRP levels reflect an acute inflammatory response, CRPM measures a fragment of the degraded protein. CRP is known to be synthesised in the liver in response to inflammation (21), and parts of it are then deposited in the inflamed tissue of the joints where to some extent it is degraded by the MMPS of the proteolytic inflammatory response (22, 23). The selected biomarkers were evaluated at baseline and one year after initiation of the selected treatment. In addition we aimed to investigate whether certain matrix turnover was descriptive of disease activity.

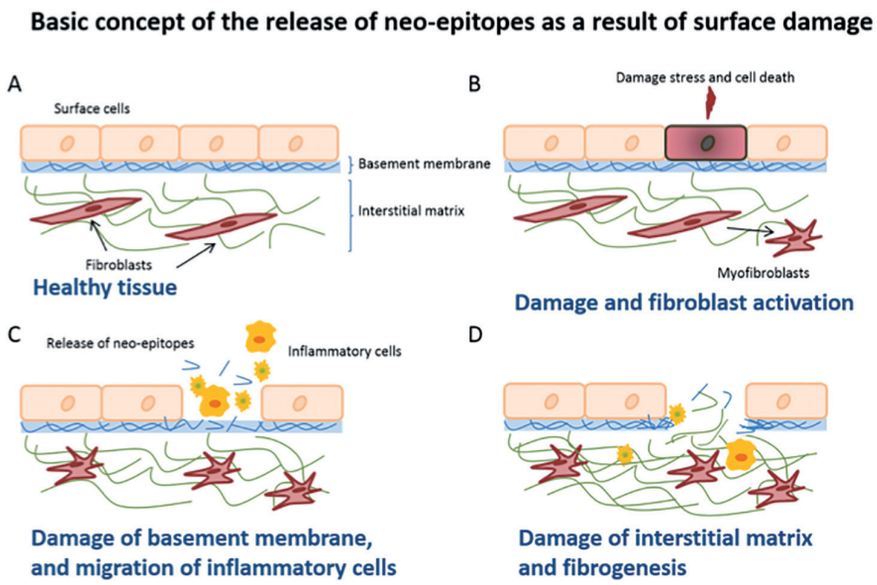


Fig. 1. Schematic representation of neo-epitopes released from the basement membrane and the interstitial tissue as a result of cell damage. **A**) Shows how healthy tissue is organised with the surface cells lying on top of the basement membrane and the interstitial tissue. **B**) Surface damage as a consequence of cell stress or death affects fibroblasts which transform to myofibroblasts and proliferate. **C**) Cell damage stimulates recruitment of inflammatory cells such as macrophages. The epithelial damage exposes the basement membrane to degradation and fragments of the basement membrane are released. **D**) As inflammation continues the interstitial matrix is exposed, activation of fibroblasts causes increased tissue remodelling which causes the release of interstitial collagen fragments and may lead to fibrogenesis.

Methods

Patients

A total of 149 Japanese patients with RA were recruited at the University Hospital of Occupational and Environmental Health (UOEH), Japan as previously described by Hirata *et al*. (24). Of these, 23 received MTX alone. 49 patients initiated ADA+MTX, another 27 initiated TOFA+MTX, and 50 initiated TCZ+MTX. Blood samples included in this study were taken at baseline prior to the first dosage of the initiated treatment and at follow-up 48–60 weeks later. Patients treated with MTX, ADA+MTX and TCZ+MTX received the drugs as part of routine clinical practice. ADA+MTX was initiated between August 2008 and March 2010; TCZ+MTX was initiated between March 2008 and October 2010, and MTX was initiated immediately after patients were diagnosed with RA between July 2011 and January 2013. Patients treated with TOFA+MTX were enrolled in domestic and global clinical phase II/III trials (trial number A3921039, A3921040 and A3921044 and an open-label extension trial A3921041) before the drug was approved by the Japanese national insurance system. TOFA+MTX was initiated between March 2008 and February 2010 and confirmed by key-open of blindness. These clinical trials were approved by the ethics committee of the UOEH (approval numbers: 10163, 10193, 10194 and 10164, respectively). Clinical parameters including tender joint count 28 (TJC28), swollen joint count 28 (SJC28), patient's global health (PGH), patient's global assessment (PGA), health assessment questionnaire (HAQ), evaluator's global assessment (EGA), CRP levels were used to calculate the disease activity score in 28 joints (DAS28) clinical disease activity index (CDAI), and simplified disease activity index (SDAI). Blood samples were taken in fasting patients at baseline and at follow-up 48–60 weeks later. In order to separate serum from whole blood, all samples were left standing for 30 minutes and then serum was separated by a centrifugal separation at 3000rpm for 10min. The collected serum was stored at -80°C and kept frozen on dry ice during transport. All patients in the MTX, TOFA+MTX and 41 of the 49 patients in the ADA+MTX group had never received a biological DMARD prior to participating in this study. Most patients in the TCZ+MTX group had previously received other kinds of biological DMARD. The study was performed in accordance with the Helsinki Declaration and with approval from the ethics committee of the UOEH (approval number: 10-114). All patients provided written informed consent.

Biomarker assessment

Serum samples were drawn and demographic information collected at baseline. Four protein biomarkers were measured both at baseline and at follow-up (1 year ± 6 weeks later). Samples were blinded and measured in duplicates using competitive enzyme-linked immunosorbent assays (ELISAs) developed by Nordic Bioscience (Herlev, Denmark).

The procedure for the assessment of C1M, C3M, C4M and CRPM have been described in detail elsewhere by Leeming *et al*. (14), Barascuk *et al*. (17), Sand *et al*. (25), and Skjot-Arkil *et al*. (23) respectively. In short 96-well streptavidin plates (Roche Diagnostics, Mannheim, Germany) coated with biotinylated synthetic peptide dissolved in assay buffer (50 mM Tris, 1% BSA, 0.1% Tween-20, pH 7.4) and incubated for 30 min at 20°C. For each assay 20 µL of peptide calibrator or sample was added to appropriate wells, followed by 100 µL of conjugated monoclonal antibody and incubated for 1–20 hour at 4°C or 20°C (depending on the specific assay). Finally, 100 µL/well of tetramethylbenzinidine (TMB) was added and the plates were incubated for 15 min at 20°C in the dark. All incubation steps included shaking at 300 rpm. After each incubation step the plate was washed five times in washing buffer (20 mM Tris, 50 mM NaCl, pH 7.2). The TMB reaction was stopped by adding 100 µL of stopping solution (0.1M sulfuric acid) and measured at 450 nm with 650 nm as the reference. Calibration curves were plotted using a 4-parametric mathematical fit model. Intra- and inter-assay variations (CV) were below 12% and 14% respectively for all of the assays. The technical specifications, of each assay, are listed in Table I.

Statistics

Mean values of summary statistics of general demographics and baseline RA characteristics are shown in Table II. Correlations were assessed by Spearman's rank tests and Bonferroni corrections were applied to adjust for multiplicity. None of the evaluated biomarkers met the criteria of normal distribution. Accordingly, baseline values were log-transformed to meet the criteria of normal distribution for intergroup comparisons. By log-transformation the biochemical marker data moderately followed a Gaussian distribution. Comparisons between the different treatment groups at baseline were performed by one-way ANOVA analysis followed by Tukeys multiple comparisons on log-transformed data. Multiple regression analysis was performed on logarithmic transformed data as well. The Wilcoxon matched pairs signed rank test was applied to compare baseline biomarker concentrations with those at follow-up. All statistical analyses were performed using MedCalc Statistical Software v. 14.8.1 and error bars on all graphs are shown as standard error of the mean (SEM).

Table I. Technical specifications of the applied assays.

	Selection peptide sequence	Standard concentrations (ng/ml)	Sample pre-dilution	Measurement range, corrected for pre-dilution (ng/ml)	Inter assay variation (%)	Intra assay variation (%)	Reference level for healthy individuals (ng/ml)
C1M	GSPGKDGVRG	233.0	1+1	20-400	6.7-13.9	2.8-8.8	24 (10.8)
C3M	KNGETGPQGP	30.0	1+3	4-88	6.6-13.1	2.0-4.1	10.2 (3.2)
C4M	ILGHVPGMLL	101.8	1+3	8.8-288	5.0-13.0	4.0-11.0	57.4 (6.9)
CRPM	KAFVFPKESD	32.7	1+3	2.0-72.0	2.2-13.3	2.2-6.0	6.4 (2.6)

Table II. Mean values and standard deviation (SD) of baseline demographic and clinical characteristics disease activity score of 28 joints (DAS28), clinical disease activity index (CDAI), simplified disease activity index (SDAI), health assessment questionnaire (HAQ), erosion score (ES), joint space narrowing (JSN), total sharp score (SHS) of the four different treatment groups: methotrexate (MTX) alone or in combination with adalimumab (ADA+MTX), tofacitinib (TOFA+MTX) or tocilizumab (TCZ+MTX).

	MTX , n=23	ADA+MTX, n=49	TOFA+MTX, n= 27	TCZ+MTX, n=50
Age, years	55.4 (13.17)	61.3 (11.1)	53.3 (12.6)	56.3 (18.5)
Male, %	21.7	12.2	14.8	12.0
Disease duration, months	6.4 (2.4)	95.5 (112)	75.8 (81.4)	153 (136)
Synthetic DMARD-naïve, %	100	0	0	0
Biological DMARD-naïve, %	100	83	100	40
DAS28	3.60 (0.9)	4.66 (1.2)	5.59 (1.2)	4.84 (1.2)
CDAI	14.3 (6.7)	24.0 (14.7)	34.9 (13.2)	25.7 (12.9)
SDAI	15.2 (7.8)	26.6 (15.1)	37.2 (14.3)	27.8 (13.8)
HAQ	0.59 (0.5)	1.11 (0.7)	1.46 (0.7)	1.46 (0.9)
ES	2.1 (3.1)	35.5 (53.9)	26.3 (31.7)	59.5 (72.3)
JSN	3.3 (4.6)	26.9 (36.1)	26.9 (26.9)	49.3 (42.6)
SHS	4.9 (7.3)	62.4 (89.5)	53.2 (57.9)	108.8 (114)
C1M, ng/ml	50.3 (32.2)	71.4 (44.2)	67 (35)	57.4 (36.6)
C3M, ng/ml	16.4 (10.4)	13.3 (6.3)	18.4 (11.4)	16.7 (10.9)
C4M, ng/ml	64.2 (24.7)	61.29 (26.7)	86.4 (34.1)	79.9 (34.4)
CRPM, ng/ml	10.0 (4.9)	11.9 (7.2)	14.5 (14.0)	15.2 (9.9)

Table III. Spearman's rank correlation coefficient (ϱ) between biomarkers and clinical data at baseline (n=149). Significance levels were set at $p<0.0026$* for the disease activity correlations and $p<0.0034$ for the remaining parameters after correction for multiplicity. *Circular error as CRP is a DAS28 parameter.

		DAS28	CDAI	SDAI	HAQ	ES	JSN	SHS
C1M	ϱ	0.40	0.24	0.34	0.23	0.17	0.13	0.15
	P	<0.0001	0.0035	<0.0001	0.0037	0.038	0.12	0.065
C3M	ϱ	0.36	0.23	0.30	0.14	0.13	0.16	0.12
	P	<0.0001	0.0042	0.0004	0.10	0.13	0.06	0.14
C4M	ϱ	0.46	0.35	0.40	0.28	0.26	0.30	0.30
	P	<0.0001	<0.0001	<0.0001	0.0007	0.0018	0.0003	0.0002
CRPM	ϱ	0.43	0.31	0.38	0.20	0.17	0.19	0.16
	P	<0.0001	0.0001	<0.0001	0.017	0.043	0.027	0.059

Results

Patient demographics

In the MTX group the mean age of patients at baseline was 55 years and the mean disease activity score (DAS28) was 3.6. In the ADA+MTX group, the mean age was 61 years and the DAS28 was 4 on average. TOFA+MTX-treated patients were on average 53.3 years old at baseline and had an average DAS28-CRP score of 5.6. The TCZ+MTX group was on average 56.3 years old with a mean DAS28 of 4.8 (Table II).

None of the patients of the MTX and TOFA+MTX groups had previously received a synthetic or biological DMARD. In the ADA+MTX and TCZ+MTX groups 41% and 20%, respectively, were synthetic-DMARD naive, and 83% and 40% were biological DMARD-naive, respectively. Patients treated with MTX alone had less structurally severe disease at baseline, as measured by erosion score (ES), joint space narrowing (JSN) and sharp score (SHS), whereas the TCZ+MTX group had markedly higher severity scores than either the TOFA+MTX or ADA+MTX group.

Baseline association between biomarkers and disease activity or severity

A one-way ANOVA analysis revealed no significant differences between the four treatment groups at baseline except for C4M levels. Patients in the ADA+MTX group had significantly lower C4M levels compared patients in the TOFA+MTX ($p=0.0014$) and TCZ+MTX ($p=0.0091$) groups but not compared to the MTX group.

The linear association between baseline biomarkers and disease activity and severity scores was evaluated by Spearman's rank correlation coefficient. All four biomarkers correlated significantly with DAS28, CDAI and SDAI (Table III). Scatterplots of the association between biomarker levels and DAS28 at baseline are shown in Figure 2. C4M levels were strongly correlated with HAQ, and there was an association between HAQ and C1M or CRPM although it was only borderline significant (p=0.0037 and p=0.017 respectively) (Table III). C4M the only evaluated biomarker which was significantly correlated with ES, JSN and SHS. Although, there was an association there were borderline significant with CRPM (p<0.05 but greater than p=0.0034) and ES, JSN and SHS.

At baseline, all biomarkers were significantly associated with disease activity (DAS28) as well as radiographic progression interpreted as SHS after adjustment for age, sex, disease duration and treatment group (Table IV).

Changes in biomarker levels between baseline and follow-up

Baseline levels of all four biomarkers were higher than the reference levels for healthy individuals (the dashed lines in Figure 3). Elevated levels of C3M, C4M and CRPM compared with healthy individuals were most pronounced in the TCZ+MTX and TOFA+MTX groups. Turnover of type I collagen, evaluated by the mean level of C1M, decreased by between 21–35% from baseline to follow-up. This decrease was significant for patients in all four treatment groups (p-values ranging from 0.009 to <0.0001) (Fig. 3A). At follow-up, C1M levels of the MTX and the TCZ+MTX groups had, on average, fallen to levels resembling those seen in healthy individuals (24ng/ml (SD 10.8)).

C3M was on average elevated at baseline in all of the treatment groups compared with healthy individuals (10.2ng/ml (SD 3.2)) (Fig. 3B). The levels of C3M significantly decreased (p-values ranging from 0.012 to <0.0001), by 16–39% for each group on average, from baseline to follow-up and were approaching the level of healthy individuals in all four treatment groups.

C4M levels significantly decreased (p-values ranging from 0.0017 to <0.0001), between 20–32% across the four treatment groups, between baseline and follow-up (Fig. 3C). The levels of C4M were close to the average level of healthy individuals (57.4 ng/ml

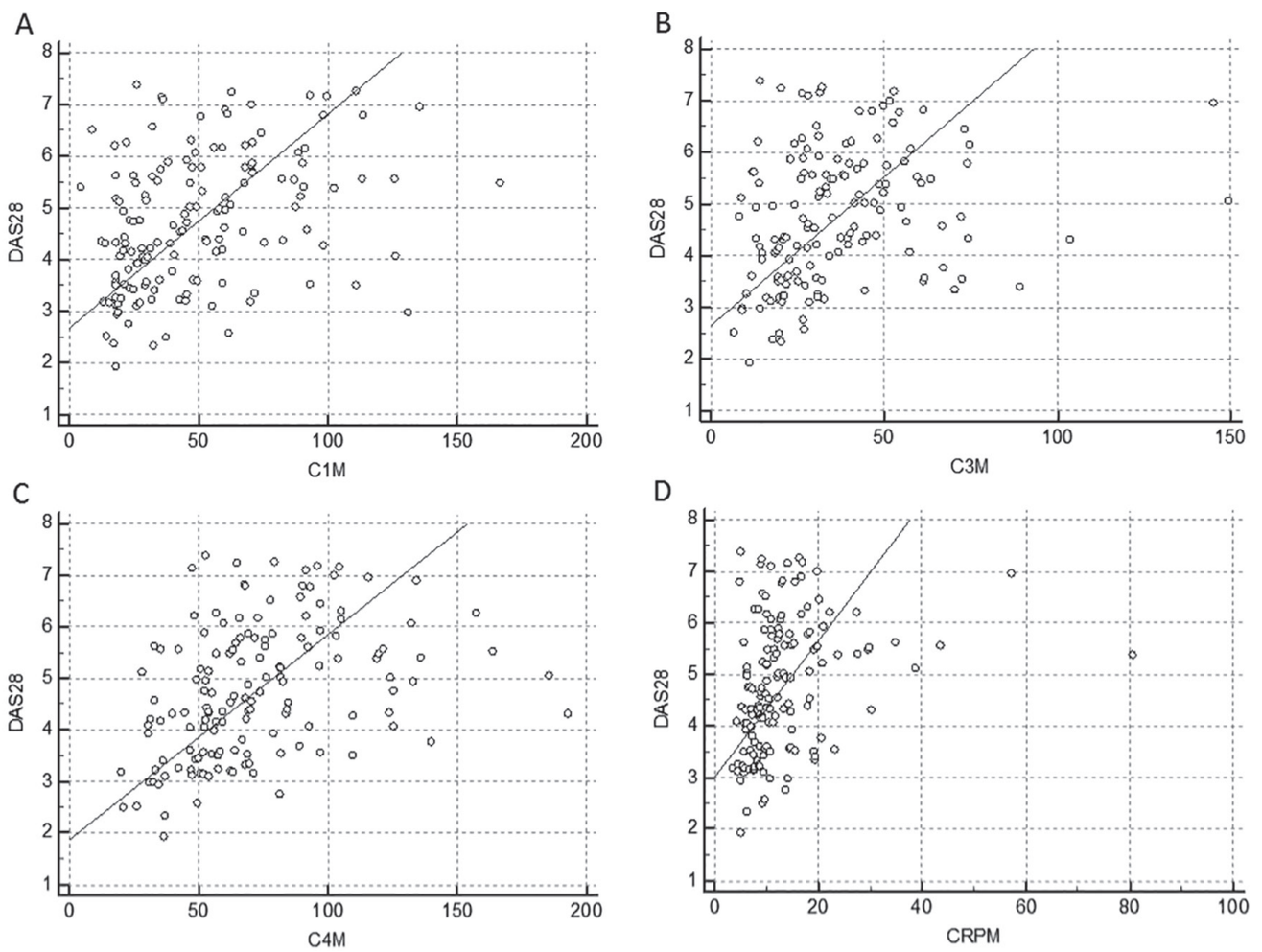


Fig. 2. Scatterplots of the association between each the four biomarkers (ng/ml) and DAS28 at baseline: A) C1M, B) C3M, C) C4M and D) CRPM. Trend lines are shown as reduced major axis lines.

Table IV. Multiple regression analysis illustrated the association between the different biomarkers and DAS28 and SHS at baseline (n=149).

	DAS28				SHS			
	n	SD	ϱ	*p*	n	SD	ϱ	*p*
C1M – Alone	148	0.35	0.37	<0.0001	149	0.209	0.18	0.029
C1M – Adjusted for age, sex and disease duration	149	0.35	0.36	<0.0001	149	0.13	0.22	0.009
C1M – Adjusted for age, sex, disease duration, and treatment groups	149	0.34	0.39	<0.0001	149	0.13	0.22	0.009
C3M – Alone	149	0.40	0.34	<0.0001	149	30.7	0.13	0.13
C3M – Adjusted for age, sex and disease duration	148	0.41	0.33	<0.0001	149	20.9	0.25	0.015
C3M – Adjusted for age, sex, disease duration. and treatment groups	148	0.40	0.33	<0.0001	149	20.8	0.20	0.018
C4M – Alone	148	0.50	0.44	<0.0001	148	40.0	0.24	0.0036
C4M – Adjusted for age, sex and disease duration	148	0.50	0.44	<0.0001	148	26.6	0.31	0.0001
C4M – Adjusted for age, sex, disease duration, and treatment groups	148	0.50	0.44	<0.0001	148	27.4	0.29	0.0004
CRPM – Alone	147	0.42	0.39	<0.0001	148	32.8	0.21	0.011
CRPM – Adjusted for age, sex and disease duration	147	0.42	0.39	<0.0001	148	33.4	0.28	0.0006
CRPM – Adjusted for age, sex, disease duration, and treatment groups	147	0.42	0.39	<0.0001	148	23.0	0.26	0.0014

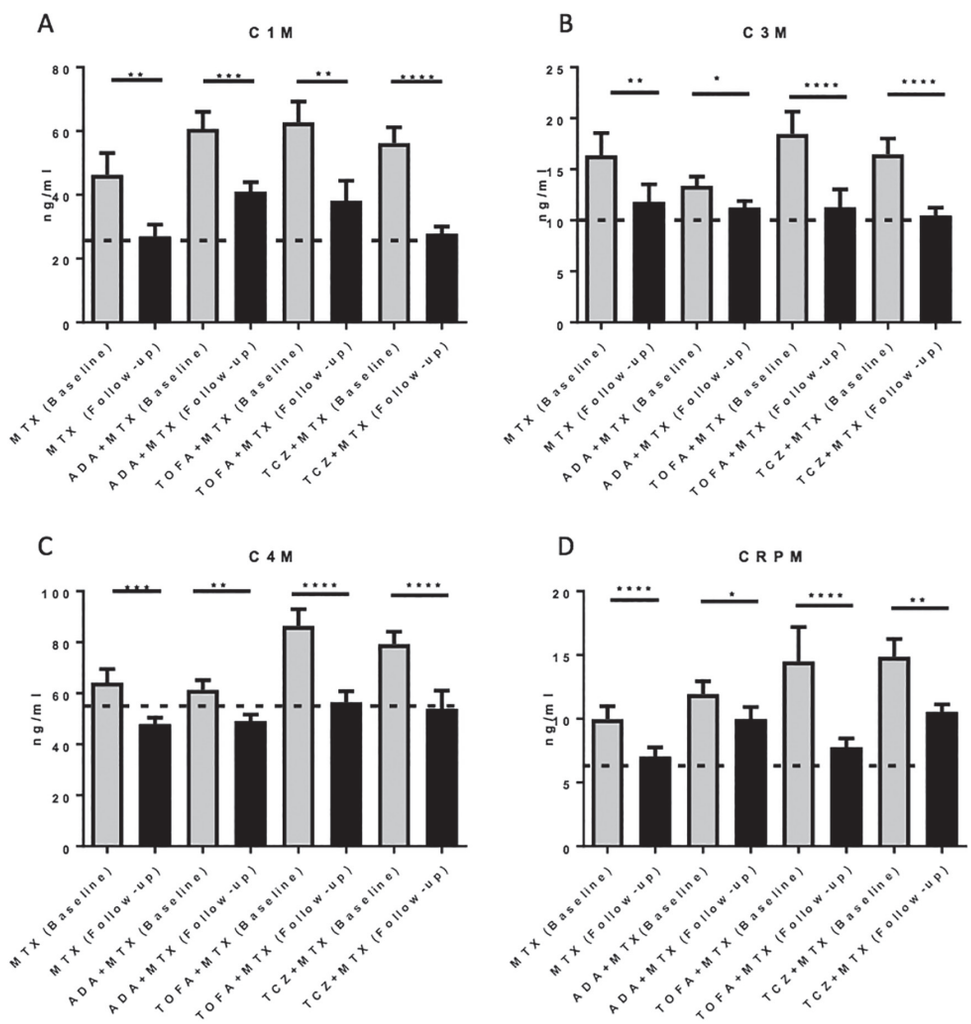


Fig. 3. Evaluations of biomarker levels at baseline and follow-up:
A: Type I collagen degradation in connective tissue evaluated by C1M.
B: Type III collagen degradation evaluated by C3M.
C: Type IV collagen degradation evaluated by C4M.
D: MMP-degraded CRP evaluated by CRPM. The dashed line represents the reference level of healthy individuals. Difference between baseline and follow-up was evaluated using the Wilcoxon matched pairs signed rank test.
**p*<0.05, ** *p*<0.01, ****p*<0.001, *****p*<0.0001.
Error bars are illustrated as SEM.

(SD 6.9)) at follow-up in all treatment groups.

For CRPM, only patients in the MTX and the TOFA+MTX group achieved levels close to the average of healthy individuals (6.4ng/ml (SD 3.2)) at follow-up (Fig. 3D). Although CRPM was significantly decreased at follow-up in all four treatment groups compared with baseline (*p*-values ranging from 0.032 to <0.0001), the levels in the ADA+MTX and TCZ+MTX groups remained 53% and 62% higher at follow-up than the reference level for healthy individuals. At baseline, the MTX group had generally lower biomarker values than the three other treatment groups.

Discussion

Chronic inflammation of RA and the damage induced in the epithelium result in exposure of the underlying tissues and subsequent remodelling of the basement membranes and interstitial tissue. In in this study some of these changes were profiled by selected biomarkers in patients at different stages of RA. The selected patient populations ranged from newly diagnosed, treatment naïve patients that were introduced to MTX at baseline to patients that were diagnosed for several years, and had been introduced to several treatments on top of MTX.

The main findings of the current study are:

- *Diagnostically*
  Basement membrane degradation (measured by C4M) and interstitial tissue degradation (C1M and C3M) were all higher than the levels for healthy individuals. The differences in these levels were more pronounced in the patient groups with the longest disease duration (TCZ+MTX and TOFA+MTX).
- *Efficacy*
  The turnover of connective tissue depicted by types I, III and IV degradation products were, significantly decreased and approaching normal levels at follow-up. In addition, the significant decrease of CRPM levels in all treatment groups suggests that inflammation of the joints was reduced.
- *Disease score*
  Significant correlations were found between all four evaluated biomarkers and severity of disease when measured as DAS28, SJC28 or SDAI. This indicates that tissue turnover is a key constituent of disease activity.

Currently, several biomarkers are used to assess autoantibodies, elevated levels of inflammatory cytokines as well as evaluation of bone and cartilage turnover (26-29). Our results do, however, indicate that it may be relevant to consider turnover of the connective tissue in addition to the current standard disease activity parameters in RA, since inflammation and tissue damage are key drivers in all autoimmune diseases including RA (30).

Inflammation-driven MMP activity leads to the release of neo-epitopes from the interstitial matrix into serum. These changes can then be evaluated by C1M and C3M, which reflect degradation of types I and III collagen, the main components of the interstitial tissue (16, 31, 32). Chronic inflammation and tissue damage affect the rate of remodelling and changes in the composition of the interstitial matrix (33), which is in line with our results of C1M and C3M levels in RA. Both C1M and C3M have previously been found to be of prognostic and diagnostic value in RA (15, 19, 20, 34), osteoarthritis (35) and ankylosing spondylitis (18, 19). In this study both markers were correlated with the disease activity parameters DAS28, CDAI and SDAI, and with the radiographic progression, even after correction for sex, age, disease duration. C1M levels were found to be elevated in all four treatment groups at baseline. At follow-up, both C1M and C3M levels in all treatment groups had decreased significantly. This indicates that modulation of the interstitial tissue is reduced in response to pharmacological intervention.

As type IV collagen is the main component of the basement membrane, we consider C4M is appropriate for the evaluation of basement membrane modulation. We found that baseline C4M levels were increased by 10.1–53.1% compared to healthy individuals on average. This is in line with previous study which reported that C4M was elevated in the other inflammatory joint disease ankylosing spondylitis (18). In addition, C4M levels of the presented study correlated with the disease activity parameters DAS28, CDAI, HAQ, JSN, as well as the radiographic (SHS) score at baseline. C4M levels approached the levels of healthy individuals at follow-up. Thus, it appears that not only is the epithelial layer, but also modulation of the underlying basement membrane, disrupted by RA, even in the newly diagnosed MTX group. The decreased levels of C4M at follow-up indicate that the basement membrane may benefit from anti-inflammatory intervention as turnover seems to have been slowed down.

The endothelium – one of the epithelial subtypes – has been found to be altered as a consequence of systemic inflammation (36, 37) and this dysfunction is present even in young RA patients with low disease activity (38). The basement membrane is also widely affected by the chronic inflammation of RA as it becomes exposed due to endothelial dysfunction (39), which may explain the increased C4M levels at baseline compared with the reference level. It has been reported that the endothelial function is improved by interventions such as MTX (40; 41), anti-inflammatory therapies such as anti-TNF-α agents (37, 42), which may explain the decreased C4M levels at follow-up.

CRPM was selected to quantify the CRP fragments released from the inflamed tissue. The biomarker has previously been shown to be upregulated in ankylosing spondylitis (23) as well as in RA (43). The four groups in this study had all highly elevated CRPM levels at baseline, which were 53–128% higher on average, compared to the reference level. Although all treatment groups had a significant decrease in CRPM levels at follow-up, only patients in the MTX and the TOFA+MTX groups reached levels close to the reference level for healthy people. The ADA+MTX and TCZ+MTX groups were still 53% and 62% elevated at follow-up compared to the reference. This may in part be due to the intergroup differences in demographics and disease parameters. The generation of CRPM depends on MMPs (23). Thus CRPM levels reflect MMP activity in presence of inflammation.

It is peculiar that the selected biomarkers although the baseline levels appeared to vary between the four treatment groups (although only significantly for C4M), they show similar patterns in response to treatments. This indicate that the initiated treatments excert the same effect on the interstitial tissue and basement membrane at least within the studied timeframe. It could also be that MTX is the main driver of this effect, since MTX is a common denominator of all four treatment groups. However, it should be noted that the patients were at different stages of disease at baseline. It was only the MTX that consisted of MTX naïve patients

at baseline, indicating that MTX alone may not be sufficient to keep the biomarker levels low if or when the disease progress.

A limitation of the study was the small size of the patient population, thus these data need to be confirmed in a larger study such as a phase III clinical trial. The intergroup differences in demographic and clinical data of the treatment groups makes it difficult to compare the treatment groups. Furthermore, the biomarker reference values are based on samples mainly from Caucasians, which may be slightly different from other populations such as those of Asian descent as the RA patients included in the study. This is however the first time that biomarkers of connective tissue destruction have been evaluated in a head-to-head treatment study.

In conclusion, remodelling of the interstitial tissue and basement membranes is elevated in RA and is associated with disease pathology. This level of tissue destruction was modulated by different types of intervention. Baseline levels of tissue destruction were associated with disease progression and could reflect central aspects of the mechanism driving disease progression.

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