IL-6 receptor inhibition modulates type III collagen and C-reactive protein degradation in rheumatoid arthritis patients with an inadequate response to anti-tumour necrosis factor therapy: analysis of connective tissue turnover in the tocilizumab RADIATE study

P. Juhl, C.S. Thudium, N.S. Gudmann, M.A. Karsdal, A.-C. Bay-Jensen, A.S. Siebuhr

Nordic Bioscience, Herlev, Denmark.

Abstract

Objective

The objective of this study was to examine the tissue degradation in response to anti-IL6 receptor treatment in rheumatoid arthritis (RA) patients which are anti-TNF-α inadequate responders.

Methods

RADIATE was a randomised, double-blinded, placebo-controlled, parallel-group, phase III trial. RA patients with previous inadequate response to anti-TNFα therapy (n=299) were randomly assigned to tocilizumab 4 or 8 mg/kg with methotrexate (10–25 mg weekly) or placebo with methotrexate. Type III collagen degradation (C3M) and CRP degradation (CRPM) were analysed in serum samples at baseline and 16 weeks.

Results

Treatment with 4 and 8 mg/kg tocilizumab significantly decreased C3M (p=0.0001 and p=0.0007) and CRPM (p<0.0001) levels after 16 weeks. Changes in C3M and CRPM levels after 16 weeks correlated well with the changes in disease activity score 28 (DAS28). Change in CRPM levels furthermore correlated moderately with the change in patient pain (VAS) ($r_{partial}$ of 0.20) and Health assessment questionnaire disability index (HAQ-DI) ($r_{partial}$ of 0.24). Changes in biomarker levels above median change led to an odds ratio of 1.95 (C3M) and 3.00 (CRPM) for achieving the American College of Rheumatology 20% improvement criteria (ACR20).

Conclusion

This study shows that a decrease in inflammation leads to a decrease in excessive extracellular matrix degradation. It furthermore supports earlier shown evidence that tocilizumab works in the treatment of RA patients, although there is a clear need for identifying and selecting patients who are more likely to respond to treatment.

Key words

biomarkers, rheumatoid arthritis, tocilizumab, anti-IL-6 receptor, tissue turnover

Pernille Juhl, MSc
Christian Schneider Thudium, PhD
Natasja Stæhr Gudmann, PhD
Morten Asser Karsdal, Professor
Anne-Christine Bay-Jensen, PhD
Anne Sofie Siebuhr, PhD

Please address correspondence to:
Dr Pernille Juhl,
Nordic Bioscience,
Biomarkers and Research,
Herlev Hovedgade 205-207,
2730 Herlev, Denmark.
E-mail: pju@nordicbio.com

Received on July 18, 2017; accepted in revised form on October 30, 2017.

Clinical trial registry number: NCT00106522.

Competing interests: none declared.

Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory disease characterised by severe joint pain, extensive inflammation of the synovial membrane (synovitis) and elevated tissue turnover. RA patients are treated with disease-modifying anti-rheumatic drugs (DMARDs). Tocilizumab (TCZ) is a biological DMARD targeting the interleukin 6 (IL-6) receptor. TCZ inhibits both soluble and membrane-bound IL-6R, thus, blocking IL-6 binding and thereby limiting its pro-inflammatory activities and decreasing prothrombotic biomarkers (1, 2). IL-6 works as part of a complex network of pro-inflammatory cytokines including TNF-α, which together promotes tissue degradation (3). IL-6 regulates a variety of cells such as leukocytes and hepatocytes and furthermore have systemic effects (4). In the liver, it stimulates the hepatocytes to increase production of acute-phase proteins such as C-reactive proteins (CRP) (4). IL-6 is also shown to play an important role in the regulation of tissue turnover (4-6); amongst other through the up-regulation of matrix metalloproteinases (MMP) (7). In high tissue turnover diseases, such as RA, CRP is deposited in the inflamed tissue, where it is susceptible to degradation by MMPs. CRPM is an MMP-degraded fragment of CRP, which is released to the circulation and can be measured as a biomarker of tissue inflammation (8). The biomarker CRPM provides information on tissue inflammation and proteolytic degradation compared to only providing information on the presence of inflammation (8). As CRP is up-regulated with inflammation, infection and tissue injury, it is considered a non-specific biomarker of general inflammation (9), whereas CRPM is a specific biomarker of tissue inflammation .

The extracellular matrix (ECM) is an important dynamic structure present in all tissues and is of great importance for tissue function and helps control cell phenotype and function (10). Healthy tissue continuously undergoes controlled remodelling mediated by proteases. Altered architecture and composition of tissues are pathological hallmarks of RA (11); the fine balance between formation and degradation of ECM has shifted. This can be seen as excessive tissue turnover of the synovial joints (12, 13) partly mediated by MMPs (14). Type III collagen is the main structural collagen of the synovium. An increased turnover of this collagen have been reported in RA (15-17), and is associated with disease activity (16, 18). C3M is an MMP-degraded fragment of type III collagen serving as a biomarker of tissue destruction (19). With this biomarker it is possible to quantify the tissue degradation, aiding in the understanding of the magnitude of tissue degradation in RA.

TCZ has demonstrated efficacy in patients with moderate to severe RA with inadequate response to methotrexate and anti-TNF in the LITHE and RADIATE studies (6, 20). A range of biomarkers were analysed in both studies: In the RADIATE trial TCZ regulate the bone markers CTX-I (cathepsin K-mediated bone resorption), N-MID (osteocalcin) and ICTP (MMP-mediated type I collagen degradation). In the LITHE study TCZ modulated markers of connective tissue turnover (21, 22). Here, it was found that TCZ decreased tissue degradation measured by biomarkers as C3M and CRPM (6) in the MTX inadequate responder population. However, patients do not always respond to the same treatments. The aim of this study was to examine the tissue degradation in response to anti-IL6R treatment in RA patients which are anti-TNF-α inadequate responders.

Patients and methods

Study design and serum samples

RADIATE (Clinical trial registry number: NCT00106522) was a randomised, double-blinded, placebo-controlled, parallel-group, phase 3 trial first described by Emery *et al*. (22). The RADIATE study was conducted throughout North America and Western Europe where patients (n=499) were randomly assigned to tocilizumab 8 mg/kg or 4 mg/kg or placebo intravenously every 4 weeks. In addition, all patient received methotrexate (10-25 mg weekly). Adults (>18 years of age) with moderate to severe active RA and failure to respond to one or more anti-TNF-α within a year were included in the study.

For this sub-study, samples from 299 consenting patients were collected for biochemical marker assays and measured blinded. Only patients with baseline and follow-up samples at week 16 were included. Samples without enough material were excluded.

All participants gave written, informed consent before inclusion in the study and was carried out in accordance with the principles of the declaration of Helsinki.

Biochemical marker assay

Serum from patients at baseline and after 16 weeks was examined in ELISAs measuring type III collagen degradation (C3M) and CRP degradation (CRPM). The C3M (19) and CRPM (23) assays are solid phase competitive ELISAs developed and produced by Nordic Bioscience (Herlev, Denmark). The assay were run according to instructions from the manufacturer.

Samples below the lower limit of detection were assigned the value of the lower limit of detection (C3M: 1.0 ng/ml, CRPM: 0.5 ng/ml)

Statistical analyses

Summary statistics were used for baseline demographics and baseline RA characteristics separated into treatment (Table I). As the data was not normally distributed, the data was transformed using the natural logarithm. Difference in biomarker levels over time was tested by paired *t*-test for each treatment. The dose dependent difference between two treatment groups TCZ4 and TCZ8, and PBO was examined by one-way ANOVA at baseline and week 16. The correlation between baseline levels of biomarkers and demographics and clinical features were examined using spearman's rank correlation while multiple linear regression were utilised to examine the correlation between biomarkers levels (baseline and change in levels) and disease activity. Bonferroni correction was used to find the critical *p*-value, which was found to be <0.005 with 10 parameters and <0.017 for 3 parameters.

All statistics analyses were performed using MedCalc v. 14.8.1 and Prism Graphpad v. 7.0 and graphs were made using Prism Graphpad v. 7.0.

Table I. Baseline demographics and disease activity shown as mean (SD). One way ANOVA was used to examine any significant difference between the treatment groups.

Characteristic	Placebo + MTX (n=65)	MTX + Tocilizumab 4 mg/kg (n=72)	MTX + Tocilizumab 8 mg/kg (n=80)
Age, years	54.1 (12.2)	51.6 (12.4)	53.1 (11.2)
Female, %	75.4	81.9	87.5
Disease duration, years	11.9 (10.2)	11.7 (9.9)	13.4 (9.3)
BMI	28.0 (6.4)	28.5 (5.9)	26.8 (6.1)
Patient pain, VAS	61.8 (23.8)	68.2 (18.1)	65.3 (20.2)
HAQ-DI score	1.7 (0.6)	1.7 (0.5)	1.7 (0.6)
DAS28	6.9 (1.0)	6.9 (0.9)	6.7 (1.0)
CRP, mg/dL	4.0 (4.4)	3.7 (4.1)	3.2 (3.5)
ESR, mm/h	57.1 (32.6)	51.7 (28.2)	47.5 (29.2)
SJC	19.3 (10.4)	19.3 (9.6)	17.5 (9.3)
TJC	33.1 (14.6)	31.7 (14.5)	30.9 (15.6)

BMI: body mass idex; CRP: c-reactive protein; DAS28: disease activity score 28 joints; ESR: erythrocyte sedimentation rate; HAQ-DI: health assessment questionnaire disease idex; SJC: swollen joint count; TJC: tender joint count; VAS: visual analogue score.

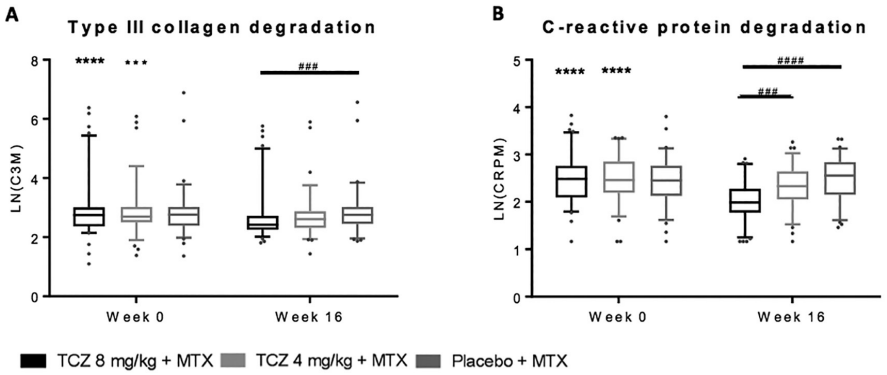

Fig. 1. Dose dependent treatment profile. Serum biomarkers were measured at baseline and week 16 in the three treatment groups and transformed using natural logarithm. Data are shown as mean with 95% confidence interval and significance are shown as *for paired *t*-test (Wilcoxon test) and as #for one way ANOVA (Kruskal-Wallis test). */#: p=0.05, **/##: p=0.005, ***/###: p=0.0005, ****/####: p<0.0001. **A**: Type III collagen degradation (C3M). **B**: CRP degradation (CRPM).

Results

Baseline demographics

The full trial description has been previously described by Emery *et al*. (22) and Karsdal *et al*. (5). The RADIATE study had 499 patients separated into three treatment arms from which this sub-study had similar distribution of gender, age, and disease duration. There were no significant difference between the three different treatment arms characteristic, neither were there any significant difference in characteristics between the full trial and this sub-study (Table I).

Biomarker levels

C3M levels were significantly decreased at week 16 compared to baseline in patients receiving TCZ8 (p<0.0001) and TCZ4 (p=0.0007). After 16 weeks, a significant difference between the TCZ8 and placebo group was observed (p=0.0002) (Fig. 1A).

TCZ8 and TCZ4 (p<0.0001) significantly decreased CRPM levels at week 16 compared to baseline. A dose dependent decrease was observed for CRPM levels as the difference between TCZ8 and TCZ4 (p=0.0007) and TCZ8 and placebo (p<0.0001) were significant different (Fig. 1B).

Correlation between baseline levels and clinical parameters

The relationship between tissue degradation and clinical parameters was examined using the natural logarithm to the biomarker baseline values (Table III).

C3M and CRPM levels were moderately correlated with disease activ-

Table II. Spearman's rank correlation between the natural logarithm to baseline levels of C3M and CRPM and baseline levels of clinical parameters.

	LN (C3M)		LN (CRPM)	
	ϱ	*p*	ϱ	*p*
Age, years	-0.04	NS	-0.06	NS
Disease duration, years	-0.07	NS	-0.04	NS
BMI	-0.08	NS	-0.02	NS
Patient pain, VAS	0.30	**<0.0001**	0.34	**<0.0001**
HAQ-DI score	0.26	**0.0001**	0.33	**<0.0001**
DAS28	0.32	**<0.0001**	0.42	**<0.0001**
CRP, mg/dL	0.51	**<0.0001**	0.66	**<0.0001**
ESR, mm/h	0.37	**<0.0001**	0.54	**<0.0001**
SJC	0.18	NS	0.28	**<0.0001**
TJC	0.11	NS	0.16	NS

BMI: body mass index; CRP: c-reactive protein; DAS28: disease activity score 28 joints; ESR: erythrocyte sedimentation rate; HAQ-DI: health assessment questionnaire disease index; SJC: swollen joint count; TJC: tender joint count; VAS: visual analogue score. Bonferroni correction was used and the critical *p*-value was <0.0005.

Table III. The natural logarithm of C3M and CRPM at baseline was correlated by multiple linear regression with changes in diseases activity scores, with and without adjustment.

	LN (C3M)		LN (CRPM)	
	p	$r_{partial}$	*p*	$r_{partial}$
ΔPatient pain, VAS	NS	0.14	**0.0026**	0.33
ΔPatient pain, VAS adjusted for gender, age, BMI and disease duration	NS	0.11	**0.0021**	0.35
ΔHAQ-DI	NS	0.25	**0.0013**	0.36
ΔHAQ-DI adjusted for gender, age, BMI and disease duration	NS	0.23	**0.0021**	0.35
ΔDAS28	NS	0.25	**0.0004**	0.39
ΔDAS28 adjusted for gender, age, BMI and disease duration	NS	0.25	**0.0010**	0.38

Δ: change in; C3M: type III collagen degradation; CRP: C-reactive protein; CRPM: C-reactive protein degradation; DAS28: disease activity score 28 joints; HAQ-DI: health assessment questionnaire disease index; VAS: visual analogue score. Bonferroni correction was used and the critical *p*-value was <0.017.

Table IV. Multiple linear regression for changes in C3M and CRPM for all three treatment arms. The changes in CRP and type III collagen degradation levels were correlates with the changes in Patient pain (VAS), HAQ-DI and DAS28.

	Δ C3M		Δ CRPM	
	p	$r_{partial}$	*p*	$r_{partial}$
ΔPatient pain, VAS	NS	0.16	**0.002**	0.21
ΔPatient pain, VAS adjusted for gender, age, BMI and disease duration	NS	0.15	**0.0038**	0.20
ΔHAQ-DI	**0.014**	0.17	**0.0002**	0.26
ΔHAQ-DI adjusted for gender, age, BMI and disease duration	NS	0.16	**0.0004**	0.24
ΔDAS28	**0.0001**	0.27	**<0.0001**	0.38
ΔDAS28 adjusted for gender, age, BMI and disease duration	**0.0001**	0.28	**<0.0001**	0.38

Δ: change in; CRP: C-reactive protein; DAS28: disease activity score 28 joints; HAQ-DI: health assessment questionnaire disease index; VAS: visual analogue score. Bonferroni correction was used and the critical *p*-value was <0.017.

ity (patient pain (VAS), HAQ-DI and DAS28) with rho values ranging from 0.26 to 0.42 ($p \leq 0.0001$). C3M and CRPM were highly correlated with CRP and ESR with rho values between 0.37 and 0.66 ($p<0.0001$). Only CRPM correlated moderately with SJC (0.28, $p<0.0001$) while neither C3M nor CRPM at baseline correlated with age, disease duration, BMI, or TJC.

To investigate the significance of tissue specific inflammation and tissue degradation at baseline with the response to treatment, baseline levels were correlated to the change in disease activity in the TCZ8 treated patients. Multiple linear regression models were applied on natural logarithmic transformed baseline levels with and without correction for gender, age, BMI, and disease duration (Table III).

Baseline levels of C3M were not associated with changes in disease activity. Baseline CRPM was associated with Δpatient pain (VAS), ΔHAQ-DI and ΔDAS28 both with and without adjustment for gender, age, BMI, and disease duration with partial correlation coefficient of minimum 0.33 ($p<0.0026$).

The association of change in C3M and CRPM with response to treatment

To examine if changes in tissue degradation and tissue specific inflammation levels represented an overall improvement in disease activity, the change in levels were correlated to the changes in disease activity scores after 16 weeks. Furthermore, the effect of gender, age, BMI and disease duration were investigated by adjusting for these parameters (Table IV).

ΔC3M and ΔCRPM levels correlated well with ΔDAS28 ($r_{partial} \geq 0.27$, $p \leq 0.0001$) with and without adjustment of gender, age, BMI and disease duration. ΔCRPM resulted in a partial correlation coefficient of 0.38 ($p<0.0001$) with ΔDAS28, independent of adjustment for gender, age, BMI, and disease duration. ΔCRPM levels furthermore correlated moderately with Δpatient pain (VAS) and ΔHAQ-DI having a partial correlation coefficient of 0.21 and 0.26, respectively. When the multiple linear regression models were adjusted for gender, age, BMI and disease duration, the $r_{partial}$ decreased to 0.20 and 0.24, respectively.

The association between change in tissue degradation levels and response to treatment was examined and depicted in Figure 2. Changes in C3M levels above median have an increased odds ratio of 1.95 for achieving ACR20 and of 2.31 for achieving ACR50 after 16

weeks compared to changes in levels below median.

A change in CRPM levels above median have an increased odds ratio of 3.00 for achieving ACR20 and 3.46 for achieving ACR50 after 16 weeks compared to patients with a CRPM level change below median.

Discussion

RA is a complex disease and the aetiology is not fully elucidated, but it is clear that the heavy inflammation in joints leads to severe tissue degradation and joint deterioration. Patients are largely administered anti-inflammatory therapy and due to the side effects associated with this type of treatment as well as the cost, early selection of the patients benefitting from a specific treatment is important. Thus, there is a need to understand the degradation of tissue that takes place in the joints and assessing if a given treatment is efficacious. Furthermore, patients do not always respond to the same treatments. It is therefore necessary to understand which patients will benefit from a given treatment and in which way the tissue is affected. In this biomarker sub-study of RADIATE, the relationship between biomarkers of tissue degradation and anti-IL6R treatment was examined to gain an understanding of tissue remodelling during treatment and the relationship between tissue remodelling and disease activity. We demonstrated that TCZ8 decreased C3M and CRPM, and there was a correlation between CRP degradation, disease activity and ACR responders.

Biomarkers, associated with tissue turnover, provide tools for accurately describing ongoing processes in high tissue turnover diseases such as RA. These tools allow investigation into the mode of action of different treatments and may provide an understanding of the role of different cytokines in the disease. Biomarkers of bone turnover have previously been examined in this cohort, however an understanding of the synovial tissue turnover is affected by anti-IL-6 receptor treatment have not been fully investigated (5).

Biomarkers of tissue turnover have great potential in quickly determining whether a treatment works on tissue inflammation. In this study the degradation of type III collagen and CRP were significantly diminished after treatment with TCZ8. Additionally, TCZ4 treatment significantly diminished CRPM and showed a tendency to decrease C3M levels. TCZ appeared to reduce tissue degradation of type III collagen and CRP in a dose dependent manner. The biomarkers used in this study have previously been used in the LITHE study where they showed similar percentage reduction in CRPM levels at week 16 relative to baseline. However, in the LITHE study a larger percentage reduction in C3M was observed compared to this study (20). The main difference between the LITHE and RADIATE studies is the inclusion criteria. Both studies enrolled moderate to severe RA patients, but in the LITHE study patients were MTX inadequate responder (21), while the RADIATE study enrolled anti-TNF-α inadequate responders (22). This suggest that patients who are anti-TNF-α inadequate responders might not be responding as well as MTX inadequate responders to TCZ. Further studies are needed to clarify if TCZ is more efficacious in patients who are only MTX inadequate responders.

The degradation products are naturally occurring and part of healthy tissue remodelling. Increased levels have, however, previously been associated with inflammation and fibrosis (19, 24). Patients enrolled in this study have moderate to severe RA, likely associated with a high degree of inflammation and an increased tissue turnover in the affected joints. It is therefore not surprising that degradation products are correlated with disease activity at baseline but not with age, disease duration and BMI. The association between the degradation of type III collagen and CRP and inflammation are not fully elucidated. It is therefore not known if the degradation products have an influence on the duration of the inflammation and on the response to treatment. In for example osteoarthritis, degradation products, such as small aggrecan degradation products are shown to

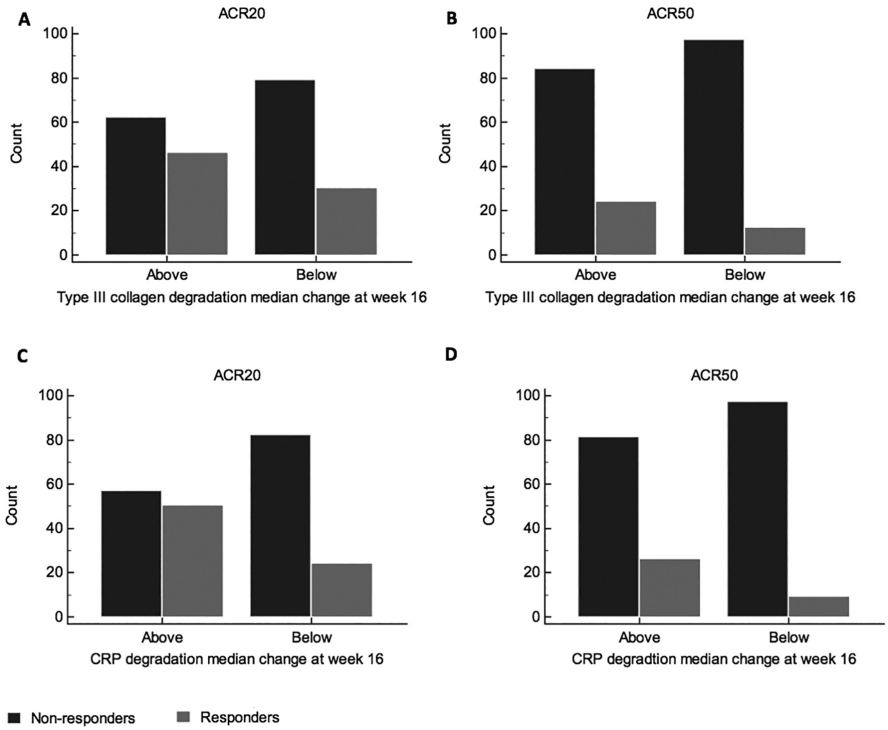

Fig. 2. Frequency of ACR20 and ACR50 responders and non-responders (week 16) with a change in type III collagen or CRP degradation above or below median. **A**) The frequency of ACR20 responders with a change in type III collagen degradation above median. **B**) The frequency of AC50 responders with a change in type III collagen degradation above median. **C**) The frequency of ACR20 responders with a change in CRP degradation above median. **D**) The frequency of AC50 responders with a change in CRP degradation above median.

elicit inflammatory responses by binding to immune receptors in joint resident cells leading to upregulation of pro-inflammatory cytokines and tissue inflammation (25).

In this study, the baseline levels of CRPM correlated well with the changes in disease activity (patient pain (VAS), HAQ-DI and DAS28). Adjustment for gender, age, BMI and disease duration did not significantly change the correlation between CRPM levels and change in disease activity indicating that these parameters are not the basis for the correlation. Baseline levels of C3M did not correlate with the change in disease activity as a response to treatment, indicating that C3M baseline levels are too homogeneous to differentiate patients and their disease activity response at baseline.

The association between the change in tissue degradation and disease activity markers were examined together with the effect of gender, age, BMI and disease duration on this association. While the type III collagen degradation correlated with the disease activity, the degradation of CRP correlated better especially with the change in DAS28. An explanation might be that DAS28 is calculated with CRP as one of the components. The treatment inhibits IL-6, which downstream inhibits CRP and further downstream CRPM. Thus, the treatment itself lowers DAS-CRP, even if the remainder of the DAS components is not affected by the treatment. Thus, the correlation between these is bias of the treatment response. However, CRP and CRPM offers different information. CRP gives information on inflammation while CRPM gives information on tissue turnover. Even though CRP correlates with CRPM, they are not measures of the same. By measuring CRPM, it is possible to assess the treatments effect on the tissue and not just the systemic inflammation.

Both changes in type III collagen and CRP degradation levels were associated with response to treatment based on ACR20 and ACR50 indicating that a change in inflammation level and thereby tissue degradation increases the chance of overall improvement. Overall, this supports the hypothesis of a close relationship between disease activity, response to treatment and inflammation in the tissue.

One of the main limitations of this study was that it was not possible to measure serum samples at 24 or 52 weeks assessing the long-term treatment response. However, Kremer *et al*. have shown that improvements after 8 weeks is maintained up to 5 years in the LITHE study (26). Another limitation is that there are no radiographics to support the findings of improvements. However, radiographic scores is known not to be very sensitive to changes in joints evaluated for a short period of time (27).

In conclusion: when patients receive anti-IL6R treatment for 16 weeks have a decrease in disease activity and achieves ACR20 or ACR50 it is the drug decreasing inflammation. The decrease in inflammation is believed to decrease the excessive degradation of extracellular matrix and other components in the tissue, which is further supported by the findings. This study clearly support that the serological markers of tissue turnover C3M and CRPM can be used as disease markers in RA regarding disease activity and response to therapy. Thus, C3M and CRPM are candidate markers for stratification of RA patients into risk categories and thereby supporting ongoing efforts to develop precision medical treatments.

References

1. JONES G, SEBBA A, GU J *et al*.: Comparison of tocilizumab monotherapy versus methotrexate monotherapy in patients with moderate to severe rheumatoid arthritis : the AMBITION study. *Ann Rheum Dis* 2010; 69.
2. GUALTIEROTTI R, INGEGNOLI F, GRIFFINI S, GROVETTI E, MERONI PL, CUGNO M: Prothrombotic biomarkers in patients with rheumatoid arthritis: The beneficial effect of IL-6 receptor blockade. *Clin Exp Rheumatol* 2016; 34: 451-8.
3. MCINNES IB, SCHETT G: Cytokines in the pathogenesis of rheumatoid arthritis. *Nat Rev Immunol* 2007; 7: 429-43.
4. CALABERASE LH, ROSE-JOHN S: IL-6 biology: implications for clinical targeting in rheumatic disease. *Nat Rev Rheumatol* 2014; 10: 720-7.
5. KARSDAL MA, SCHETT G, EMERY P *et al*.: IL-6 receptor inhibition positively modulates bone balance in rheumatoid arthritis patients with an inadequate response to anti-tumor necrosis factor therapy: biochemical marker analysis of bone metabolism in the Tocilizumab RADIATE study (NCT00106522). *Semin Arthritis Rheum* 2012; 42: 131-9.
6. BAY-JENSEN AC, PLATT A, SIEBUHR AS, CHRISTIANSEN C, BYRJALSEN I, KARSDAL MA: Early changes in blood-based joint tissue destruction biomarkers are predictive of response to tocilizumab in the LITHE study. *Arthritis Res Ther* 2016: 7-15.
7. KOTHARI P, PESTANA R, MESRAOUA R *et al*.: IL-6-mediated induction of matrix metalloproteinase-9 is modulated by JAK-dependent IL-10 expression in macrophages. *J Immunol* 2014; 192: 349-57.
8. SKJØT-ARKIL H, SCHETT G, ZHANG C *et al*.: Investigation of two novel biochemical markers of inflammation, matrix metalloproteinase and cathepsin generated fragments of C-reactive protein, in patients with ankylosing spondylitise. *Clin Exp Rheumatol* 2012; 30: 371-9.
9. HIRSCHFIELD GM, PEPYS MB: C-reactive protein and cardiovascular disease: new insights from an old molecule. *Q J Med* 2003; 96: 793-807.
10. KARSDAL MA, NIELSEN MJ, SAND JM *et al*.: Extracellular matrix remodeling: the common. *Assay Drug Dev Technol* 2013: 70-92.
11. BONNANANS C, CHOU J, WERB Z: Remodelling the extracellular matrix in development and disease. *Nat Rev Mol Cell Biol* 2014; 15: 786-801.
12. FIRESTEIN GS: Evolving concepts of rheumatoid arthritis. *Nature* 2003; 423: 356-61.
13. KAROUZAKIS E, NEIDHART M, GAY RE, GAY S: Molecular and cellular basis of rheumatoid joint destruction. *Immunol Lett* 2006; 106: 8-13.
14. BURRAGE PS, MIX KS, BRINCKERHOFF CE: Matrix metalloproteinases: Role in arthritis. *Front Biosci* 2006; 11 (October 2016): 529-43.
15. MAIJER KI, GUDMANN NS, KARSDAL MA: Neo-epitopes - fragments of cartilage and connective tissue degradation in early rheumatoid arthritis and unclassified arthritis. *PLoS One* 2016; 11.
16. BAY-JENSEN AC, WICHUK S, BYRJALSEN I *et al*.: Circulating protein fragments of cartilage and connective tissue degradation are diagnostic and prognostic markers of rheumatoid arthritis and ankylosing spondylitis. *PLoS One* 2013; 8.
17. SIEBUHR AS, WANG J, KARSDAL M, JIN Y, ZHENG Q: Matrix Metalloproteinase-dependent turnover of cartilage, synovial membrane, and connective tissue is elevated in rats with collagen induced arthritis. *J Transl Med* 2012; 10: 1.
18. SIEBUHR A, BAY-JENSEN AC, LEEMING DJ *et al*.: Serological identification of fast progressors of structural damage with rheumatoid arthritis. *Arthritis Res Ther* 2013; 15: 1-9.
19. BARASCUK N, VEIDAL SS, LARSEN L *et al*.: A novel assay for extracellular matrix remodeling associated with liver fibrosis: An enzyme-linked immunosorbent assay (ELISA) for a MMP-9 proteolytically revealed neo-epitope of type III collagen. *Clin Biochem* 2010; 43: 899-904.
20. BAY-JENSEN AC, PLATT A, BYRJALSEN I, VERGNOUD P, CHRISTIANSEN C, KARSDAL MA: Effect of tocilizumab combined with

methotrexate on circulating biomarkers of synovium, cartilage, and bone in the LITHE study. *Semin Arthritis Rheum* 2014; 43: 470-8.

21. KREMER JM, BLANCO R, BRZOSKO M *et al*.: Tocilizumab inhibits structural joint damage in rheumatoid arthritis patients with inadequate responses to methotrexate: results from the double-blind treatment phase of a randomized placebo-controlled trial of tocilizumab safety and prevention of structu. *Arthritis Rheum* 2011; 63: 609-21.
22. EMERY P, KEYSTONE E, TONY HP *et al*.: IL-6 receptor inhibition with tocilizumab improves treatment outcomes in patients with rheumatoid arthritis refractory to anti-tumour necrosis factor biologicals: results from a 24-week multicentre randomised placebo-controlled trial. *Ann Rheum Dis* 2008; 67.
23. SKOJT-ARKIL H, SCHETT G, ZHANG C *et al*.: Investigation of two novel biochemical markers of inflammation, matrix metalloproteinase and cathepsin generated fragments of C-reactive protein, in patients with ankylosing spondylitis. *Clin Exp Rheumatol* 2012; 30: 371-9.
24. BAY-JENSEN AC, LEEMING DJ, KARSDAL MA: Ankylosing spondylitis is characterized by an increased turnover of several different metalloproteinase-derived collagen species: a cross-sectional study. *Rheumatol Int* 2012; 32: 3565-72.
25. FOSANG AJ, LAST K, GARDINER P, JACKSON DC, BROWN L: Development of a cleavage-site-specific monoclonal antibody for detecting metalloproteinase-derived aggrecan fragments: detection of fragments in human synovial fluids. *Biochem J* 1995; 310 (Pt 1: 337-43.
26. KREMER JM, BLANCO R, HALLAND A-M *et al*.: Clinical efficacy and safety maintained up to 5 years in patients with rheumatoid arthritis treated with tocilizumab in a randomised trial. *Clin Exp Rheumatol* 2016; 34: 625-33.
27. SOKKA T: Radiographic scoring in rheumatoid arthritis: a short introduction to the methods. *Bull NYU Hosp Jt Dis* 2008; 66: 166-8.