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Decrease in immunoglobulin free light chains in patients with rheumatoid arthritis upon rituximab (anti-CD20) treatment correlates with decrease in disease activity

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► Additional data are published online only. To view these files please visit the journal online (<http://ard.bmj.com>).

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ABSTRACT

Objectives Immunoglobulin (Ig) free light chains (FLCs) are short-lived B cell products that contribute to inflammation in several experimental disease models. In this study, FLC concentrations in inflamed joints of patients with rheumatoid arthritis (RA) as compared to patients with osteoarthritis were investigated. In addition, the relationship of FLCs and disease activity upon B cell depletion (rituximab) in patients with RA was studied.

Methods Synovial fluid (SF) and tissue from patients with RA were analysed for local presence of FLCs using ELISA and immunohistochemistry. In addition, FLC concentrations were measured (at baseline, 3 and 6 months after treatment) in 50 patients with RA with active disease who were treated with rituximab. Changes in FLCs were correlated to changes in disease activity and compared to alterations in IgM, IgG, IgA, IgM-rheumatoid factor (RF) and IgG-anti-citrullinated protein antibody (ACPA) concentrations.

Results FLCs were detected in synovial tissue from patients with RA, and high FLC concentrations were found in SF from inflamed joints, which positively correlate with serum FLC concentrations. Serum FLC concentrations significantly correlated with disease activity score using 28 joint counts, erythrocyte sedimentation rate (ESR) and C reactive protein, and changes in FLC correlated with clinical improvement after rituximab treatment. Moreover, effect of treatment on FLC concentrations discriminated clinical responders from non-responders, whereas IgM-RF and IgG-ACPA significantly decreased in both patient groups.

Conclusions FLCs are abundantly present in inflamed joints and FLC levels correlate with disease activity. The correlation of FLC concentrations and disease activity indicates that FLCs may be relevant biomarkers for treatment response to rituximab in patients with RA and suggests that targeting FLC may be of importance in the therapy of RA.

the importance of B cells and their products have been well recognised at present.⁵ Administration of B cell depleting antibody directed to CD20 (rituximab) to patients with RA results in profound and longstanding depletion of peripheral CD20 B cells in most patients. In a significant number of patients, this leads to a decrease in disease activity.^{6,7} Several mechanisms through which B cells can contribute to inflammation are proposed, like antigen presentation to T cells, and production of proinflammatory cytokines, chemokines and autoantibodies (eg, rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA)),⁸ which can trigger immune complex-mediated responses.^{9,10} Although these autoantibodies decline after rituximab treatment, this does not consistently correlate with clinical response.^{11,12} Since a clear understanding of the working mechanism of rituximab is lacking, it cannot be excluded that B cell products other than cytokines and autoantibodies are important in mediating disease at the local and systemic level. One of these putative other factors are κ and λ immunoglobulin (Ig) free light chains (FLCs). FLCs are produced physiologically by B lymphocytes, plasmablasts and/or plasma cells. It has been shown that FLCs can exhibit several biological activities like enzymatic activity, complement activation, specific binding activity to antigens and binding to different cell types, including mast cells.¹³ Serum FLC concentrations are demonstrated to be increased in several inflammatory diseases including asthma, rhinitis, multiple sclerosis, inflammatory bowel disease and RA.¹³⁻¹⁷ In RA, serum FLC concentrations correlate with disease activity.^{14,18}

In the present study, we demonstrate increased local and systemic concentrations of FLC in patients with RA. In addition, we document that downregulation of FLCs by rituximab is associated with clinical improvement.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disorder featured by inflammation of synovial tissue, characterised by proliferation of synoviocytes, production of proinflammatory mediators and subsequent infiltration of predominantly mononuclear cells.^{1,2} In chronically inflamed synovial tissue, macrophages, T cells, B cells, plasma cells and mast cells are abundantly present. Although RA has often been considered predominantly a T cell mediated macrophage-dependent disease,^{3,4}

PATIENTS AND METHODS

Patients and treatment protocol

To measure local FLC concentrations in the joint, synovial fluid (SF) was collected from 68 randomly selected patients with a clinical diagnosis of RA according to American College of Rheumatology (ACR) criteria,¹⁹ and 24 patients with osteoarthritis (OA). Paired serum samples were collected at the same time from a subgroup of these patients with RA (11 patients). To assess presence of FLCs in

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joint tissue, synovial tissue was collected from eight randomly selected patients with RA and patients with OA that underwent total knee joint replacement.

To measure the effect of rituximab treatment on serum FLCs, another 50 patients with RA were included in an open study with a follow-up of 6 months. Patients were eligible for enrolment if they were 18 years of age or older, had a clinical diagnosis of RA according to ACR criteria¹⁹ and failed treatment with combination(s) of disease-modifying antirheumatic drugs and/or tumour necrosis factor (TNF)-blocking agents. Patients were excluded when one of the following criteria was present: life expectancy of less than 6 months, severe uncontrolled infections, irreversible major organ dysfunction, HIV positivity, a positive pregnancy test or unwillingness to use adequate contraception for the duration of the study. The patients were treated with two infusions of 1000 mg rituximab (Roche, Woerden, The Netherlands) at day 1 and day 14. Patients were assessed for disease activity using the Disease Activity Score in 28 joints (DAS28)²⁰ before the start of treatment, and 3 and 6 months after start of treatment. Efficacy end point was the response according to European League Against Rheumatism (EULAR) criteria.²¹ At similar time intervals, blood was collected for the assessment of serum antibody titres, including FLCs, and the inflammatory parameters erythrocyte sedimentation rate (ESR) and C reactive protein (CRP). In addition, serum FLC concentrations were analysed in a non-atopic control group (n=14). All patient material collection was performed according to the local Medical Ethical Committees of the University Medical Center, Utrecht and the Academic Medical Center, Amsterdam, and all patients gave their informed consent before participation in the study.

Measurements of serum antibody titres

Total serum IgG, IgM and IgA titres were measured by immunoturbidimetry on the COBAS Integra 400/700/800 (Roche Diagnostics, Indianapolis, Indiana, USA) and nephelometry on the Image 800 (Beckman Coulter, Fullerton, California, USA) according to the manufacturer's guidelines. Serum titres of anti-cyclic citrullinated protein antibodies of the IgG isotype (IgG-ACPA) were measured using a commercial ELISA (Immunoscan RA, mark 2; Euro-Diagnostica, Arnhem, The Netherlands), according to the manufacturer's instructions and as previously reported.^{22 23} Serum titres of RF of the IgM isotype (IgM-RF) were measured using a standardised ELISA, as previously described.^{23 24} Total serum or SF FLC concentrations were determined using an ELISA adapted from Abe *et al*^{25 26} (see supplementary material for details).

Immunohistochemistry

Cryostat tissue sections were allowed to air dry at room temperature prior to tissue fixation using cold acetone for 10 min. After subsequent air drying and washing with phosphate buffered saline (PBS), sections were blocked with PBS-Tween/3% bovine serum albumin (BSA)/3% normal goat serum for 1 h, followed by over night incubation with the following primary antibodies diluted in blocking buffer: mouse anti-human κ FLC (F κ -C8) and mouse anti-human λ FLC (F λ -G9) (both obtained from Dr A Solomon, University of Tennessee, Knoxville, Tennessee, USA), mouse anti-human CD138 (clone MI15; Dako Cytomation, Heverlee, Belgium) and rabbit anti-human CD20 (clone BV11; Abcam, Cambridge, UK). From each tissue, three serial sections were (double) stained for λ FLC and CD20, CD138, and κ FLC and CD20. After washing in PBS-T, tissue was incubated with Alexa Fluor 568 goat anti-mouse IgG

or Alexa Fluor 488 goat anti-rabbit IgG (both Invitrogen, Breda, The Netherlands) for 1 h. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI; Invitrogen). Sections were viewed with an Eclipse TE2000-U inverted microscope (Nikon, Lijnden, The Netherlands). Images were analysed using NIS elements BR 2.3 software (Nikon).

Statistical analysis

Differences in SF FLC concentrations between patients with RA and patients with OA were determined using a Mann-Whitney test for unpaired data. Correlations between serum and SF FLCs, serum FLC concentrations and ESR and CRP, and between changes in these parameters after treatment, were determined by Spearman correlation coefficient. Changes in the concentrations of FLC, IgM, IgM-RF and IgG-ACPA after treatment were analysed by a one-way analysis of variance with repeated measures after log-transformation of the data. Sphericity was tested using Mauchly's test. Greenhouse-Geisser correction was applied when sphericity could not be assumed. Student t test was used to analyse whether different methotrexate or prednisone treatment dosages influenced FLC concentrations at baseline. p Values were considered significant when $p < 0.05$. All analyses were performed using SPSS V.15 (SPSS, Chicago, Illinois, USA).

RESULTS

Clinical and demographic features

Clinical and demographic features of all analysed patient groups are shown in table 1.

FLCs are abundantly present in SF and in synovial tissue of patients with RA

Serum FLC concentrations were significantly increased in patients with RA compared to healthy individuals. Using our standard ELISA, mean (\pm SEM) serum concentrations of healthy controls were 23.9 ± 2.18 mg/litre (κ) and 20.1 ± 2.16 mg/litre (λ) (data not shown), whereas concentrations in patients with RA were 56.3 ± 6.17 and 53.5 ± 5.31 mg/litre, respectively (see below). SF FLC concentrations were highly increased in patients with RA, compared to patients with OA (figure 1A, mean \pm SEM for κ and λ FLC concentrations in OA and RA were 24.6 ± 2.37 and 161.2 ± 33.2 mg/litre, respectively, and 27.8 ± 2.62 and 121.4 ± 17.6 mg/litre, respectively). Interestingly, maximum concentrations were as high as 1512 and 823.3 mg/litre for κ and λ FLC, respectively.

In addition, κ and λ FLC positive cells were detected within the synovial tissue of patients with RA. Double staining of FLC and CD20 revealed that, despite a few exceptions, B cells are not positive for FLC (figure 1B,D). We did not observe profound FLC expression and plasma cell infiltration in synovial tissue from clinically stable patients with OA. Staining of serial tissue sections demonstrated that FLC positive cells and CD138 positive plasma cells are present in similar regions (figure 1B–D).

SF FLC concentrations correlate with serum FLC concentrations and inflammatory parameters

SF FLC concentrations highly correlated with serum FLC concentrations (κ and λ : $r = 0.98$ and $r = 0.94$, respectively, $p < 0.0001$) (figure 2A,B). SF FLC concentrations were always higher than serum FLC concentrations when serum FLC concentrations in patients with RA exceeded those of healthy controls (figure 2C,D), suggesting local production of FLCs. In addition, high local FLC concentrations correlated with high ESR (n=48,

Table 1 Baseline patient characteristics of analysed patient groups

	Patients treated with RTX	Control group	RA (SF analysis*)	OA (SF analysis)
Demographics				
No. of patients	50	14	67	24
Female, no. (%)	36 (72)	7 (50)	49 (73)	15 (63)
Age, median (range), years	58 (22–84)	45 (23–73)	61 (26–80)†	62 (44–83)
Disease status				
Disease duration, median (range), years	12 (1–50)		19 (2–55)†	5 (2–34)
ESR, mean (\pm SD) mm/h	43.7 (\pm 27.0)		50.8 (\pm 40.7)†	NA
CRP, mean (\pm SD) mg/litre	35.2 (\pm 30.2)		47.8 (\pm 65.3)†	NA
DAS28, mean (\pm SD)	6.5 (\pm 1.1)		NA	NA
RF+ (%)	41 (82)		41 (61)	–
IgM-RF, mean (\pm SD) IU/ml	205.1 (\pm 310.6)		NA	NA
IgG-ACPA, mean (\pm SD) IU/ml	1172 (\pm 2268)		NA	NA
Medications				
Number of previous DMARDs, median (range)	5 (2–10)		NA	
Number of previous biological agents, median (range)	2 (0–4)		NA	
Biologicals (aTNF n=9, Anak n=1, RTX n=5)			9 (13)	
Methotrexate, n (%)	41 (82)		29 (43)	
Methotrexate dosage, median (range) mg/week	15 (5–30)		NA	
Other DMARDs (HChl n=6, Sul n=2, Lef n=6, Aur n=1)			17 (20)	
No DMARDs			9 (10)	
Corticosteroids, n (%)	34 (68)		23 (34)	
Prednisone dosage, median (range) mg/day	10 (5–15)		NA	

*Average (\pm SD) age, disease duration, ESR and CRP of these 12 patients were 57.2 (9.4), 16.2 (15.9), 29.8 (24.6) and 17.8 (8.8), respectively.

†Paired samples of synovial fluid and serum were analysed from 12 patients.

ACPA, anti-citrullinated protein antibody; aTNF, anti-TNF; Anak, anakinra; Aur, auromyose; CRP, C reactive protein; DAS28, disease activity score based on 28 joints; DMARDs, disease-modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; HChl, hydroxychloroquine; Lef, leflunomide; NA, not available; OA, osteoarthritis; RA, rheumatoid arthritis; RF, rheumatoid factor; RTX, rituximab; Sal, salazospirine; Sul, sulfasalazine; TNF, tumour necrosis factor.

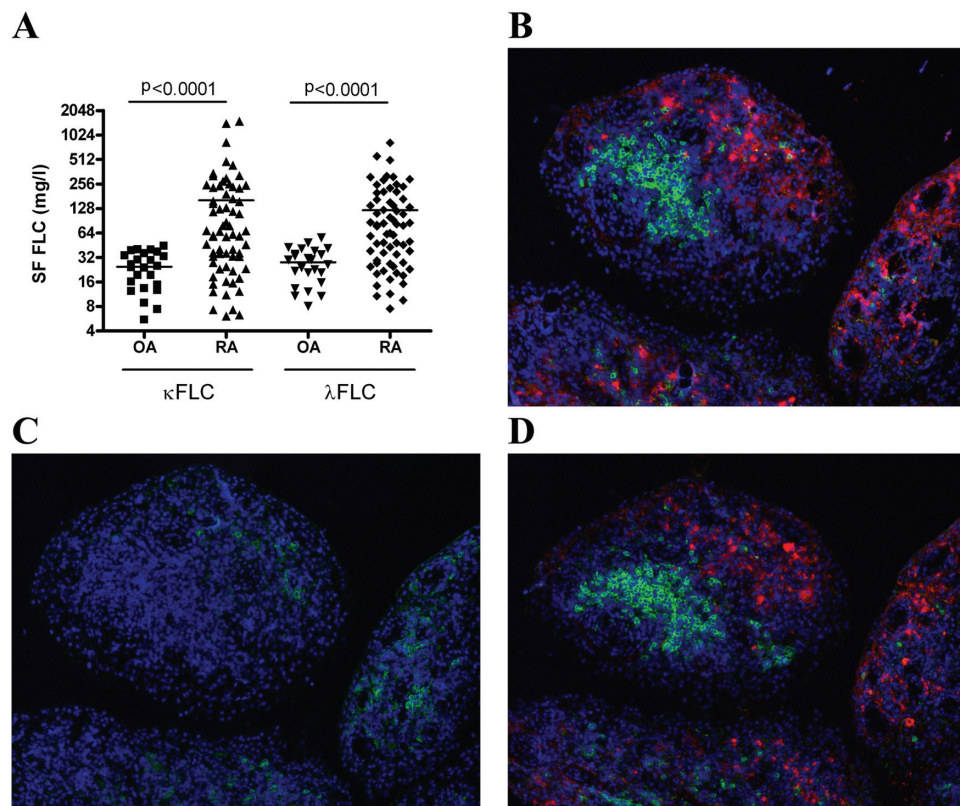


Figure 1 Immunoglobulin free light chains (FLCs) are abundantly present in the synovial fluid of patients with rheumatoid arthritis (n=67) compared to patients with osteoarthritis (n=24). A. Within the synovial tissue, λ and κ FLC positive cells were detected (B and D, respectively; red), which were not CD20 B cells (B and D; green). Staining of sequential tissue sections indicated that a great number of FLC positive cells colocalise with plasma cells (C; green).

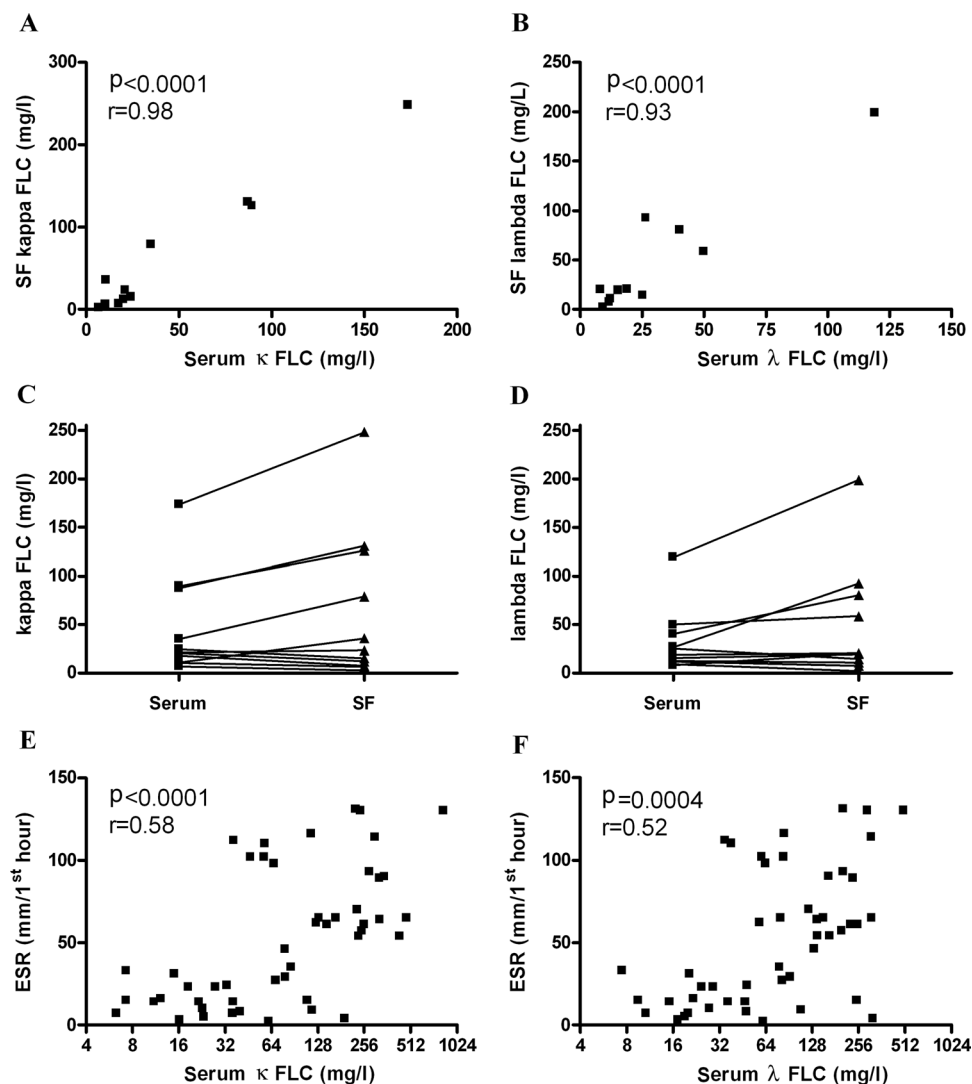


Figure 2 Local immunoglobulin free light chain (FLC) in synovial fluid (SF) highly correlates with systemic FLC concentrations for κ (A) and λ (B) in patients with rheumatoid arthritis (n=11). Paired serum and SF κ and λ FLC concentrations of these individual patients are shown in (C) and (D), respectively. SF κ FLC (mg/l) and SF λ FLC (mg/l) both correlated with the systemic inflammatory parameter erythrocyte sedimentation rate (n=48) (E and F).

κ : $r=0.58$, $p<0.0001$ and λ : $r=0.52$, $p=0.0004$ (figure 2E,F) and CRP levels (n=31, κ : $r=0.63$, $p=0.0001$ and λ : $r=0.49$, $p=0.0048$; data not shown).

Relationship between serum FLCs and disease activity markers at baseline

At baseline, serum κ and λ FLC concentrations positively correlated with disease activity scores (DAS28; κ : $r=0.38$, $p=0.0059$ and λ : $r=0.30$, $p=0.035$). Similarly, FLCs significantly correlated with ESR (κ : $r=0.54$, $p<0.0001$ and λ : $r=0.47$, $p=0.0006$) and CRP (κ : $r=0.40$, $p=0.0041$ and λ : $r=0.39$, $p=0.0052$) (figure 3).

Decrease of serum FLC concentrations after rituximab treatment correlates with disease reduction

At baseline, mean serum κ and λ FLC concentrations were 56.3 ± 6.17 and 53.5 ± 5.31 mg/litre (mean \pm SEM), respectively. κ and λ FLC concentrations significantly decreased as compared to baseline at 3 months (41.6 ± 4.35 mg/litre, $p<0.001$ and 42.7 ± 4.33 mg/litre, $p<0.01$, respectively) and 6 months (44.0 ± 4.83 mg/litre, $p<0.001$ and 43.0 ± 4.03 mg/litre, $p<0.001$, respectively) after rituximab treatment (figure 4A). In addition,

FLC concentrations significantly decreased over time in patients that responded (good to moderate) according to EULAR response criteria (κ : $-28.6\pm 4.2\%$ and $-22.4\pm 6.9\%$, $p<0.0001$, and λ : $-23.9\pm 3.1\%$ and $-18.1\pm 7.5\%$, $p<0.0001$, at 3 and 6 months, respectively). By contrast, FLC concentrations in non-responders were not significantly decreased (κ : $-0.2\pm 11.4\%$ and $+10.5\pm 16.5\%$, $p=0.72$ and λ : $+5.8\pm 15.3\%$ and $+10.2\pm 21.3\%$, $p=0.91$, at 3 and 6 months, respectively) (figure 4B,C).

Besides the association between clinical efficacy based on the EULAR response criteria and changes in FLC concentrations, a decrease in serum FLC concentrations also correlated with a decrease in ESR at 3 months after start of treatment (κ : $r=0.47$, $p=0.0006$ and λ : $r=0.40$, $p=0.0045$) (figure 5A,C), as well as 6 months after start of treatment (κ : $r=0.54$, $p<0.0001$ and λ : $r=0.45$, $p=0.0012$) (figure 5B,D). Comparable correlations were found between changes in FLC and changes in CRP (κ : $p=0.0047$, $r=0.40$ and λ : $p=0.027$, $r=0.32$ at 6 months after treatment).

Different dosing of prednisone and/or methotrexate was not associated with significant changes in FLC concentrations (data not shown). Furthermore, baseline serum FLC concentrations did not predict the degree of change in FLC concentrations, nor clinical outcome after rituximab treatment.

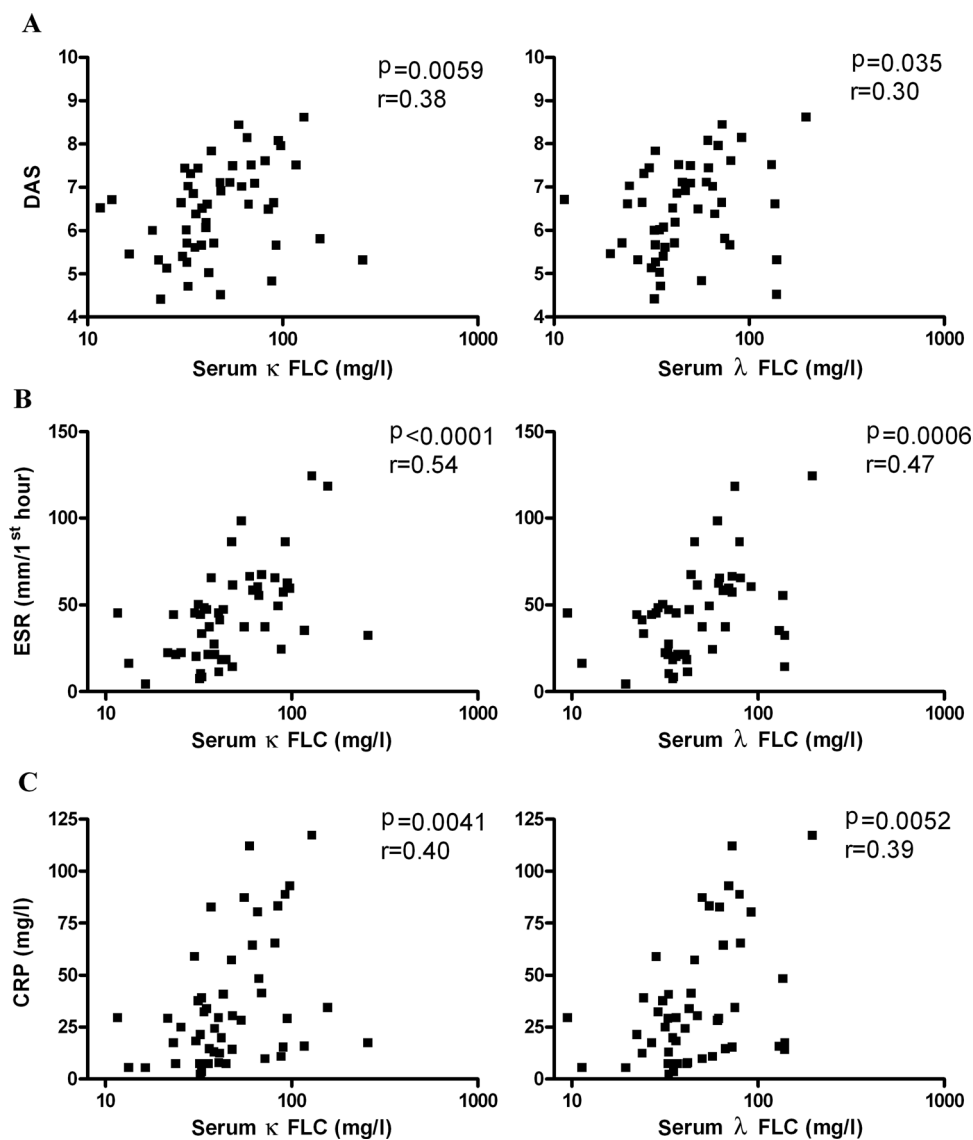


Figure 3 κ and λ immunoglobulin free light chain (FLC) serum concentrations correlate with disease activity markers before the start of rituximab treatment. κ and λ FLC concentrations both correlated significantly with (A) Disease Activity Score, (B) erythrocyte sedimentation rate and (C) C reactive protein. p Values and corresponding r values are shown in the graphs (n=50).

Effects of rituximab treatment on total Ig levels, IgG-ACPA and IgM-RF

Alterations in serum IgM, IgG and IgA concentrations were analysed and compared to changes seen for serum FLCs. The decrease of total IgM after treatment was comparable to FLC, $18.1 \pm 3.2\%$ and $19.5 \pm 2.7\%$, 3 and 6 months after treatment, respectively. In contrast to FLCs however, total IgM clearly decreased in good to moderate and non-responder patients ($p < 0.0001$ and $p = 0.017$, respectively) (see supplementary material). Total concentrations of IgG and IgA only slightly changed after 3 ($1.95 \pm 2.5\%$ and $0.83 \pm 2.0\%$) and 6 months ($2.73 \pm 2.3\%$ and $3.79 \pm 2.1\%$), and remained within their normal range.

Concentrations of the antigen-specific arthritis-related antibodies IgG-ACPA and IgM-RF, were significantly different from baseline after treatment (both $p < 0.0001$) (see supplementary material). IgG-ACPA concentrations significantly decreased over time in good to moderate responders ($p = 0.0001$) not in non-responders ($p = 0.66$). IgM-RF concentrations also significantly decreased over time in good to moderate responders ($p < 0.0001$) and in non-responders ($p = 0.016$) (see supplementary

material). Because patient data were only analysed when all data from three timepoints were available, the number of non-responding patients became limited due to missing data at single timepoints. If all non-responding patient sera at 6 months after treatment were included, IgG-ACPA also significantly decreased compared to baseline values in non-responders (IgG-ACPA: $p = 0.035$, $n = 15$).

DISCUSSION

In this study, we show that patients with RA have high concentrations of FLC in SF of affected joints and abundant expression of FLCs in the synovial tissue. Furthermore, increased serum FLC concentrations significantly correlated with SF FLC concentrations, disease activity and markers of inflammation (ESR and CRP). B cell depletion by rituximab treatment resulted in decreased serum FLC concentrations only in patients with RA that respond to therapy.

FLCs are produced and secreted by B cells, plasmablasts and/or plasma cells. We found considerable numbers of FLC-positive cells present in synovial tissue of patients with RA. A

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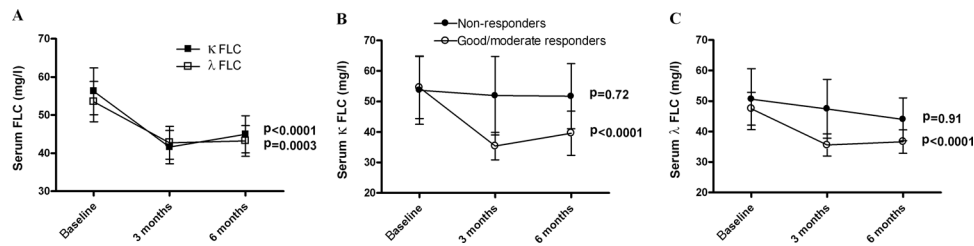


Figure 4 Changes in serum concentrations of κ and λ immunoglobulin free light chains (FLC) are associated with clinical response to rituximab. A. Total FLC concentrations significantly declined over time after rituximab treatment compared to baseline. Values are mean \pm SEM. When comparing changes in FLC concentrations at 3 and 6 months versus baseline in good to moderate (n=23) and non-responders (n=13) independently, a significant decrease in κ (B) and λ (C) FLC concentrations was only found in the good to moderate responding group. Values are mean \pm SEM.

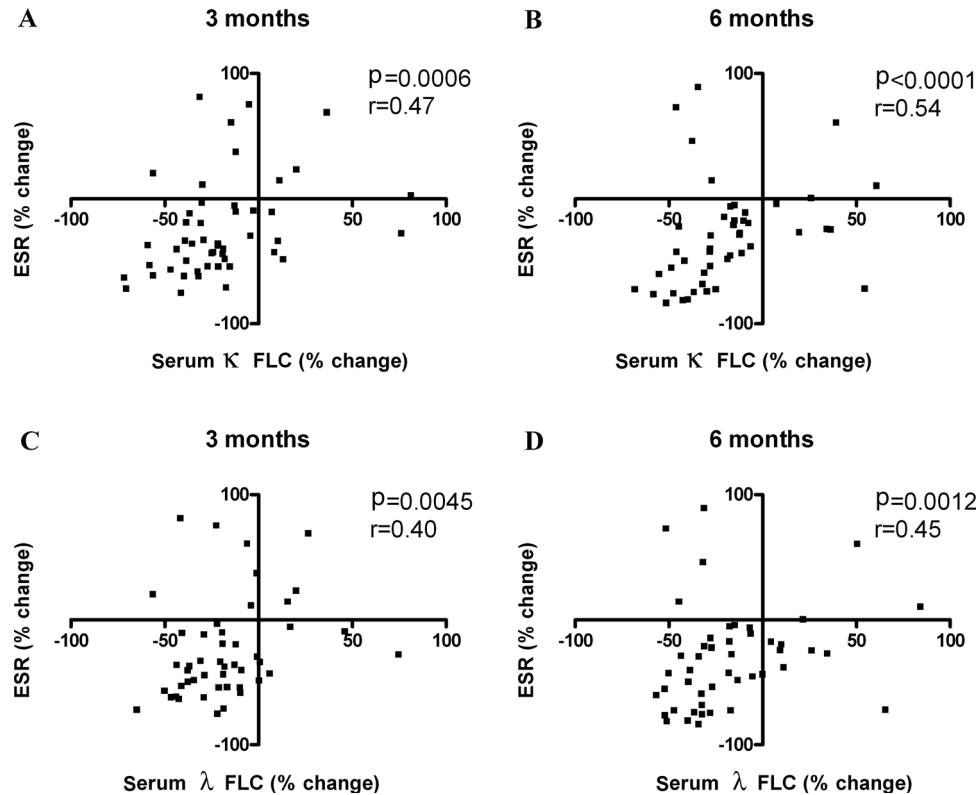


Figure 5 Changes in κ and λ immunoglobulin free light chain (FLC) concentrations correlate with changes inflammatory markers upon rituximab treatment. At 3 and 6 months after the start of treatment, a decrease in κ (A and B, respectively) and λ (C and D, respectively) FLC concentrations correlated significantly with a decrease in erythrocyte sedimentation rate (n=50). p Values and corresponding r values are shown in the graphs.

substantial proportion of these cells colocalised with CD138 plasma cells, but not CD20 cells. Recently, we found comparable colocalisation in mucosal tissue from patients with rhinitis.¹⁶ The number of FLC-positive cells appears to exceed the number of CD138 cells, suggesting that other cells in addition to plasma cells are positive for FLC. Additional to the substantial number of FLC-positive cells in RA synovial tissue, FLC concentrations were greatly increased in SF of patients with RA as compared to patients with OA, in which we did not observe profound FLC expression and synovial plasma cell infiltration. Moreover, serum FLC concentrations highly correlated with SF FLC concentrations. Together, our data indicate that FLCs are abundantly produced within the inflamed synovium of patients with RA, and that changes in serum FLC concentrations reflect changes in local FLC concentrations.

Serum FLC concentrations in patients with RA were also greatly increased compared to healthy controls. The clear correlation of both FLC subtypes and DAS28 and CRP values are

consistent with previous findings which also showed increased concentrations of serum FLCs in patients with RA which correlated with severity of disease and CRP.^{14 18 27} In addition to stronger correlations between κ and λ FLCs and CRP, in this study we find clear correlations between both FLC subtypes and ESR.

Rituximab therapy eliminates peripheral CD20-positive B lymphocytes (mature and pre-B cells) without dramatic effects on plasma cells. Peripheral B cell depletion in patients with RA significantly decreased total serum FLC concentrations. Interestingly, only good to moderate responding patients showed a significant decrease of κ and λ FLC serum concentrations at 3 and 6 months after initiation of rituximab treatment compared to baseline. Serum FLC concentrations in the good to moderate responders did not always return to values found in healthy subjects, which may be explained by a continued local production in the synovial tissues. Synovial B cells and plasma cells are found in a substantial number of patients with RA, and

the reduction of CD20 B cells after rituximab treatment in tissue is much more variable compared to the (nearly complete) depletion in peripheral blood.^{23 28} Moreover, even multiple cycles of anti-CD20 treatment does not return patients to an immune state in which all past cellular remnants of memory and autoimmune responses have been erased.²⁹

Interestingly, in contrast to the effects of rituximab treatment on serum FLC concentrations, no significant changes were found in serum FLC concentrations 6 and 12 weeks after anti-TNF treatment (n=20, data not shown). This suggests that the changes seen in FLC concentrations after rituximab treatment may be part of the therapeutic action of rituximab and are not a general feature of clinical response to disease treatment.

Since plasma cells represent end-differentiated B-lineage cells and seem to be the predominant producers of FLCs, B cell depletion is likely to affect plasma cell numbers in the long term and thereby FLC production. Synovial plasma cell numbers do not differ between clinical responders and non-responders at baseline. However, a direct relationship between the decrease in synovial plasma cells and clinical improvement over time after rituximab treatment is reported.²⁸ Therefore, indirect depletion of synovial plasma cells may be an important mechanism by which rituximab decreases FLC concentrations. This may also include depletion of a proportion of CD38 CD138 plasmablasts, which are short-lived plasma cell precursors. Interestingly, changes in FLCs do not appear to reflect changes in total IgG and IgA, nor autoantibody concentrations. Which cell type is the major producer of FLCs and how FLC expression is regulated at a molecular level is unknown at this moment. However, our data suggest that possibly different types of plasma cells produce complete (auto)antibodies and FLCs.

Parallel to the divert FLC responses in clinical responders versus non-responders, we found clear correlations between changes in FLC concentrations and ESR and CRP. Together this indicates that the percentage change in total κ and λ FLC serum concentrations may be an interesting measurement for the clinical response to rituximab therapy. In contrast, changes in RF and ACPA do not, or to a lesser extent, correlate with response to therapy, even though RF and ACPA are good prognostic biomarkers for RA development and progression at baseline.^{12 18 30 31} Our data suggest that other B cell derived factors may be involved in RA pathology and changes in FLC may reflect changes in disease activity and treatment efficacy.

Future experiments have to reveal whether FLCs with antigen specificity for synovial tissue components, such as citrullinated proteins, the Fc portion of IgG, collagen type II and proteoglycans can be identified. This can be of significant importance since we have shown previously that FLCs can mediate antigen-specific mast cell activation.³² Mast cells are suggested to play an essential role in RA pathology. Increased numbers are detected in the synovial tissue and mast cell mediators can be found in SF of patients with RA. Moreover, associations were found with proinflammatory cytokines and chondrolytic enzymes at sites of cartilage erosion.^{33–35} It is tempting to speculate that FLCs could contribute to inflammation and immunopathology by triggering antigen-specific mast cell activation in affected joints.

In conclusion, we show that increased serum FLC concentrations observed in patients with RA correlate with increased SF FLC concentrations, disease activity and inflammatory parameters. Besides, clinical response to rituximab treatment is accompanied with a significant reduction in serum FLC concentrations. Based on these data, we propose that changes in serum FLC

concentrations, to a greater extent than IgM-RF and IgG-ACPA, could serve as a biomarker for the clinical response to rituximab therapy in RA. Further studies are needed to fully explore the role of (antigen-specific) FLCs in the pathogenesis of RA and its potential as therapeutic target in the treatment of disease.

Ethics approval This study was conducted with the approval of the Ethics Committee Utrecht and Amsterdam.

Provenance and peer review Not commissioned; externally peer reviewed.

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