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Clinical response, pharmacokinetics, development of human anti-chimaeric antibodies, and synovial tissue response to rituximab treatment in patients with rheumatoid arthritis

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ABSTRACT

Objectives: To analyse whether persistence of synovial B lineage cells and lack of clinical response to rituximab treatment in patients with rheumatoid arthritis (RA) are associated with low rituximab serum levels and anti-rituximab antibody (ARA) formation.

Methods: Fifty-eight patients with RA were treated with rituximab. The clinical response was determined 24 weeks after each treatment course using the Disease Activity Score evaluated in 28 joints (DAS28) and EULAR response criteria. Rituximab serum levels, ARAs and synovial B lineage cell numbers were determined before and after treatment.

Results: Four weeks after treatment rituximab serum levels were highly variable. Low rituximab levels were associated with ARA formation (in five patients (8.6%)) and high baseline erythrocyte sedimentation rate. Interestingly, serum rituximab levels were not related to persistence of synovial B lineage cells or clinical response. Furthermore, response to treatment and re-treatment was similar in ARA-positive and ARA-negative patients.

Conclusion: There is clear variability in serum levels after rituximab treatment, but rituximab levels are not lower in patients with persistence of synovial B lineage cells or lack of clinical response. The current treatment schedule suffices to induce and maintain a clinical response, even when ARAs are formed.

Rituximab is an effective treatment for rheumatoid arthritis (RA).[1 2] Recent studies have shown that rituximab induces an incomplete B-cell depletion in the synovial tissue of a subset of patients with RA[3–6] and that persistence of synovial B lineage cells and (small numbers of) B-cell subsets in the peripheral blood is associated with lack of clinical response.[7–9] This might theoretically be explained by suboptimal rituximab levels in these patients due to a high initial B-cell load, early formation of anti-rituximab antibodies (ARAs) or other factors influencing pharmacokinetics. Therefore, we analysed the relationship between these parameters in a cohort of patients with RA starting rituximab treatment. The data were confirmed in an independent cohort.

PATIENTS AND METHODS

Patients

Patients were included from two studies on the synovial tissue response to rituximab in RA that were reported previously.[5 6] Patients had active RA (Disease Activity Score evaluated in 28 joints (DAS28[10] ⩾3.2) despite methotrexate treatment. The study protocol was approved by the ethics committee of the participating centres; all patients gave written informed consent.

Treatment regimen

Patients were treated with two infusions of 1000 mg rituximab (days 1 and 15). Pre-medication with methylprednisolone was omitted in the Academic Medical Centre/University of Amsterdam (AMC) cohort.[4] In both cohorts the DAS28 was obtained at baseline and after 24 weeks. A clinically significant decrease in disease activity was defined according to the EULAR response criteria.[11] Patients were re-treated after at least 24 weeks.[12]

Measurement of rituximab levels and ARAs

Rituximab levels and ARAs were measured after 4, 12 and 24 weeks (Leiden University Medical Centre (LUMC)) or 4, 16 and 24 weeks (AMC) (for method, see online supplemental file).

Immunohistochemistry

Synovial biopsy specimens were collected by arthroscopy in 17 patients of the LUMC cohort and 24 patients of the AMC cohort as described previously.[6 7] In the AMC cohort frozen sections were stained with anti-CD19 (Becton Dickinson, San Jose, California, USA) and anti-CD22 (CLB-B-ly; Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands) to detect B cells and anti-CD138 (clone B-B4; Immunotech, Marseille, France) to detect plasma cells. In the LUMC cohort paraffin-embedded sections were stained with anti-cytoplasmic CD20 (clone L26) to detect B cells, anti-human CD79a (clone JCD117, both from Dako, Heverlee, Belgium) for B and plasma cells, and anti-human CD138 (clone B-B4; Serotec, Oxford, UK) to detect plasma cells. The results of immunohistochemical staining were quantified using digital image analysis (AMC) or semiquantitative evaluation (LUMC[5 6]), respectively. The relationship between CD20+ B cells and rituximab levels was only analysed for baseline samples, since rituximab bound to CD20 might interfere with the detection of B cells using anti-CD20.[13]

Table 1 Patient characteristics and clinical response

Demographics (n = 58)	AMC (n = 30)	LUMC (n = 28)
Female, n (%)	24 (80)	20 (71)
Baseline disease status		
IgM-RF positive, n (%)	25 (83)	24 (86)
ACPA positive, n (%)	27 (90)	23 (82)
DAS28, mean (SD)	6.5 (1.1)	6.0 (1.2)
ESR (mm/h), median (range)	37 (4–86)	46 (5–139)
CRP (mg/dl), median (range)	29 (1.9–112)	25 (2.0–114)
Medication		
Concomitant methotrexate, n (%)	30 (100)	21 (75)
Concomitant leflunomide, n (%)	0 (0)	1 (4)
Corticosteroids, n (%)	21 (70)	11 (39)
Clinical response 24 weeks after course 1 (n = 58)	**AMC (n = 30)**	**LUMC (n = 28)**
DAS28, mean (SD)	5.0 (1.9)	4.5 (1.2)
EULAR good (%)	4 (13)	7 (25)
EULAR moderate (%)	15 (50)	17 (61)
EULAR none (%)	11 (37)	4 (14)
Clinical response 24 weeks after course 2 (n = 47)	**AMC (n = 22)**	**LUMC (n = 25)**
DAS28, mean (SD)	4.5 (1.7)	3.9 (1.1)
EULAR good (%)	5 (23)	9 (36)
EULAR moderate (%)	10 (45)	14 (56)
EULAR none (%)	7 (32)	2 (8)

ACPA, anti-citrullinated peptide antibodies; AMC, Academic Medical Centre/University of Amsterdam; CRP, C-reactive protein; DAS28, Disease Activity Score 28-joint assessment; ESR, erythrocyte sedimentation rate; EULAR, European League Against Rheumatism; IgM-RF, IgM rheumatoid factor; LUMC, Leiden University Medical Centre.

Statistical analysis

Student paired t tests were used to evaluate the change in DAS28 after treatment. Univariate linear and univariate logistic regression analyses were calculated, where appropriate, to assess the relationship first, between baseline patient characteristics, ARAs and rituximab levels; second, between rituximab levels and persistence of synovial B lineage cells; and third, between rituximab serum levels, ARAs, and clinical response determined by the decrease in DAS28 and the EULAR response (moderate/good versus none).

RESULTS

Patient characteristics

Clinical response to the first and second treatment course was available for a total of 58 and 47 patients, respectively. Table 1 shows the clinical characteristics and clinical response.

Variability in serum levels of rituximab and predictors of variability

Rituximab levels measured 4 weeks after the first infusion were remarkably variable with a range of 0.3–362 (median 110) μg/ml (fig 1, left). ARAs were detectable in two patients who had received methylprednisolone and in three who did not receive this pre-medication. Since the incidence of ARA formation was low, the two cohorts were combined, when possible, for further analyses involving ARAs. Rituximab levels in ARA-positive patients were lower than in ARA-negative patients, from 4 weeks after treatment ($p = 0.003$, $p = 0.096$, $p = 0.001$ and $p<0.001$ after 4, 12, 16 and 24 weeks, respectively; fig 1, right).

Baseline erythrocyte sedimentation rate negatively predicted rituximab levels at week 4 in both patient cohorts (AMC cohort: $r^2 = -0.17$, $p = 0.018$; LUMC cohort: $r^2 = -0.23$, $p = 0.007$); in the AMC cohort similar trends were also found for baseline C-reactive protein and DAS28 (for C-reactive protein: $r^2 = -0.23$, $p = 0.006$; for DAS28: $r^2 = -0.13$, $p = 0.032$). However, no relationship was found between rituximab levels and the presence of synovial B cells (present in 82% of patients (AMC cohort) and in 62% of patients (LUMC cohort)), synovial CD138+ plasma cells (in, respectively, 82% and 71% of patients), synovial CD79a+ B/plasma cells (in 86% of patients (only the LUMC cohort)) or numbers of CD19+ B cells in peripheral blood (data not shown). Furthermore, no relationship was found between rituximab levels after 4 weeks and body surface area, gender, use of oral prednisolone, dosage of methotrexate, or use of methylprednisolone pre-medication.

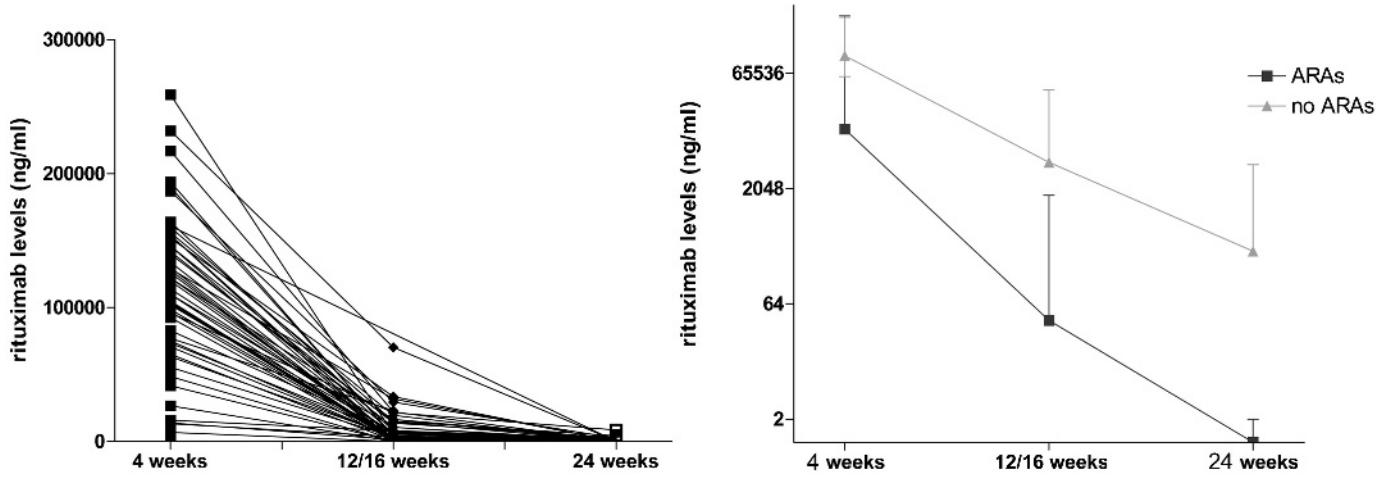

Figure 1 Rituximab levels were measured in two cohorts comprising a total of 58 patients with rheumatoid arthritis, starting rituximab treatment (left, rituximab levels after treatment in the combined cohorts). Anti-rituximab antibodies (ARAs) were detectable in five patients. The relationship between rituximab levels and ARAs was calculated for the combined cohorts, since the incidence of ARAs was low. Rituximab levels in these patients were significantly lower from 4 weeks after treatment (right). Data are represented by geometric means and 95% confidence intervals; *$p<0.05$. While rituximab clearance was highly variable, neither ARA formation nor rituximab serum levels were related to the clinical response at week 24.

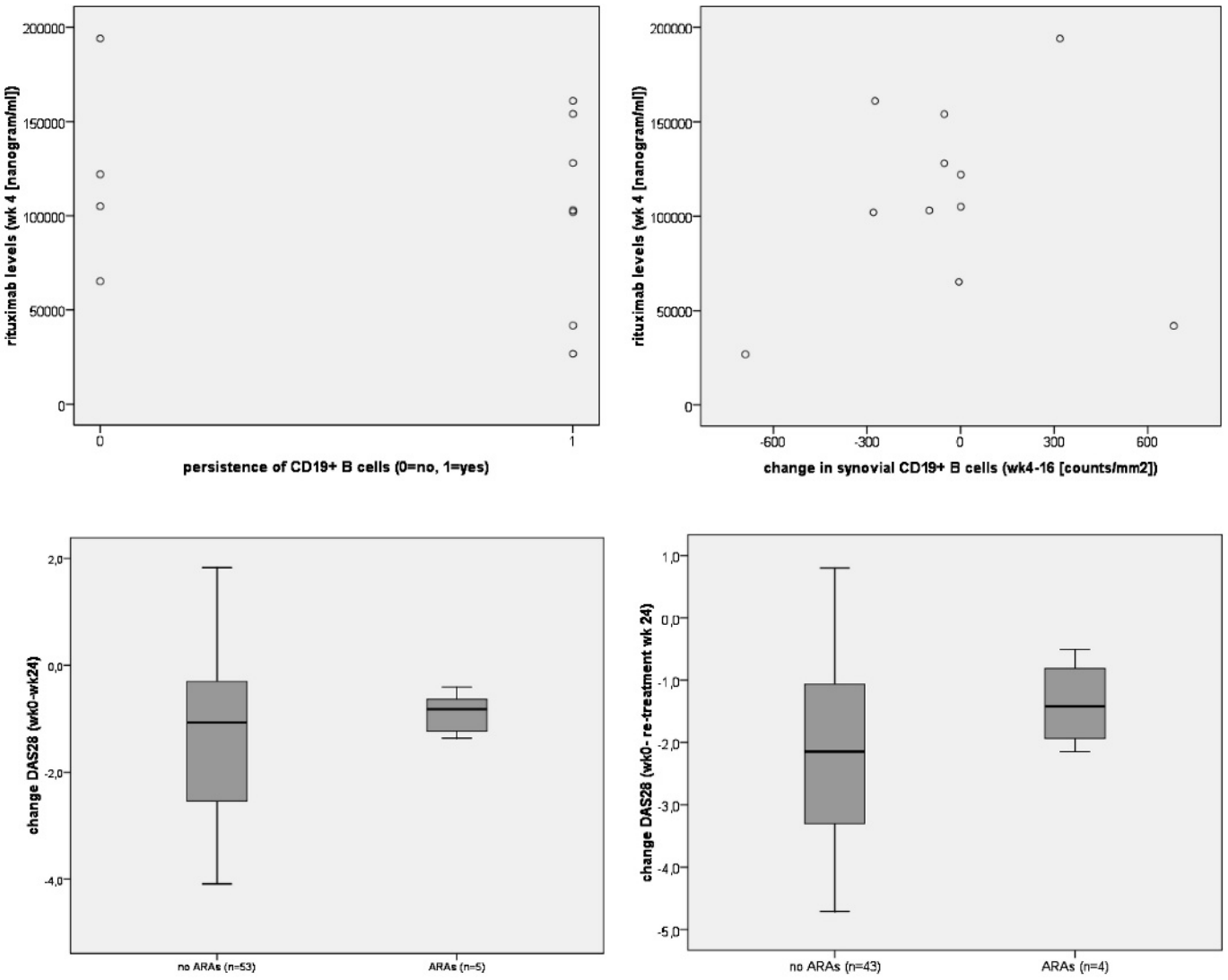

Figure 2 Analysis of the relationship between the persistence of synovial B cells and rituximab levels and influence of anti-rituximab antibody (ARA) formation on the clinical response. At week 4 (2 weeks after the second infusion) rituximab levels were similar in patients with persistence of synovial B cells and in those without detectable synovial B cells (top left, CD19+ B cells in the Academic Medical Centre/University of Amsterdam (AMC) cohort; data shown for patients with synovial B cells at baseline). Rituximab levels also did not differ between patients with a subsequent decrease or persistence of synovial B cells (top right, CD19+ B cells in AMC cohort; data shown for patients with synovial B cells at baseline). The relationship between ARAs and clinical response was calculated for the combined cohorts, since the incidence of ARAs was low. In patients who formed ARAs, clinical response to a first (bottom left) and a second treatment course (bottom right) did not differ from the response in patients without ARAs.

Table 2 Prediction of decrease in synovial B lineage cells by rituximab (RTX) levels at week 4

	RTX levels week 4
Persistence of CD22+ B cells at week 4*	0.10
Persistence of CD19+ B cells at week 4*	0.53
Change in CD22+ B cells weeks 4–16*	0.45
Change in CD19+ B cells weeks 4–16*	0.70
Change in CD138+ plasma cells weeks 4–16*	0.43
Change in CD79+ B/plasma cells weeks 0–12†	0.20
Change in CD138+ plasma cells weeks 0–12†	0.98

Logistic regression analysis was used to calculate the relationship between rituximab levels and persistence of synovial B cells at week 4; linear regression analysis was used to calculate the relationship between rituximab levels at week 4 and the subsequent change in synovial B lineage cells.
*Academic Medical Centre/University of Amsterdam; †Leiden University Medical Centre.

Synovial B cells persist despite detectable rituximab levels in peripheral blood

In the AMC cohort the change in synovial CD19+ and CD22+ B cells was analysed 4 and 16 weeks after initiation of treatment. A marked decrease in synovial B cells was found 4 weeks after the first infusion. While in some patients a further decrease in B cells occurred, at the group level B cells did not decrease further.

Synovial B cells persisted in a subset of patients (in 47% and 35% of patients after 4 and 16 weeks, respectively). We compared serum rituximab levels in patients with persistence of synovial B cells at week 4 with those in patients without detectable synovial B cells at that time point (ie, 2 weeks after the second infusion when therapeutically active levels of rituximab are expected). Of interest, serum rituximab levels did not differ between these groups (supplementary table; fig 2,

top left). Similarly, the rituximab levels at week 4 did not predict whether synovial B cells persisted or decreased further after 16 weeks (fig 2, top right). Also, rituximab levels at week 4 did not predict the persistence of plasma cells at week 16.

These data were confirmed in the LUMC cohort. Rituximab levels at weeks 4 or 12 did not correlate with persistence of synovial CD79+ B cells or CD138+ plasma cells (table 2).

Variability in rituximab levels and ARA formation are not related to the clinical response to rituximab

Consistent with the results presented above clinical non-responders did not have lower rituximab levels than responders (AMC: $p = 0.81$, $p = 0.33$ for weeks 4 and 16; LUMC: $p = 0.58$, $p = 0.11$ for weeks 4 and 12). ARA-positive patients experienced a decrease in DAS28 and EULAR response 24 weeks after the first and second treatment course similar to that of ARA-negative patients ($p = 0.87$ and $p = 0.32$, for the response to courses 1 and 2, respectively; fig 2, bottom, left and right).

DISCUSSION

We examined whether persistence of synovial B lineage cells and lack of clinical response are related to low rituximab serum levels. We show that ARA formation and differences in baseline disease activity are partly responsible for a marked variability in serum rituximab levels after treatment. Nevertheless, patients with ARAs or relatively low rituximab levels experience, on average, a depletion of synovial B lineage cells and a clinical response similar to those of patients without ARAs or higher serum levels of rituximab.

The relationship between rituximab levels, ARAs and systemic inflammation is in line with earlier observations in patients treated with infliximab.[14] Conceivably, patients with high systemic inflammation have a higher B-cell load, although we found no direct correlation with synovial or circulating B-cell numbers. Alternatively, (therapeutic) antibodies might be cleared more rapidly in these patients.

The data suggest that persistence of B cells after rituximab may be explained by expression of local survival factors rather than suboptimal rituximab levels. Furthermore, the current rituximab treatment regimen results in drug levels that remain in the therapeutic range (defined by response as indicated by clinical signs and symptoms) even when patients form ARAs. These findings are in line with two dose-ranging studies that showed no statistically significant difference in ACR20, ACR50, or ACR70 response between patients treated with 2×500 mg and those treated with 2×1000 mg rituximab.[1 15] It should be noted that the group of ARA-positive patients was relatively small and that higher serum levels might perhaps result in a clinical response of longer duration. This study was not designed to examine this possibility, since all patients were re-treated after 24 weeks if DAS28 was ⩾3.2.[12] Other limitations include the lack of data on rituximab levels at earlier time points and data on drug levels in the synovium. Although the data suggest that perhaps lower doses of rituximab might be used in some patients, it is obviously too early to recommend this for clinical practice until more data on the effects of both clinical signs and symptoms and structural outcomes become available. Moreover, there is a clear need for the identification of biomarkers that may help to further optimise rituximab treatment in individual patients.

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This publication reflects only the author's views; the European Community is not liable for any use that may be made of the information herein.

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Correction

R M Thurlings, O Teng, K Vos, *et al*. Clinical response, pharmacokinetics, development of human anti-chimaeric antibodies, and synovial tissue response to rituximab treatment in patients with rheumatoid arthritis. *Ann Rheum Dis* 2010;**69**:409-412. The published name of the author O Teng is incorrect, the correct name should read, Y K Teng.

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Notes