Progression of structural damage is not related to rituximab serum levels in rheumatoid arthritis patients

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Abstract

Objective. The most cost-effective dosing regimen for rituximab treatment in RA is currently unknown. The objective of this study is to determine whether low rituximab serum levels are associated with progression of structural damage in RA patients.

Methods. Sixty-two RA patients were treated with rituximab in three different centres. Structural damage was assessed on radiographs of hands and feet before and 1 year after therapy using the Sharp van der Heijde scoring method (SHS). Patients were divided into progressors vs non-progressors based on different cut-off values. Rituximab serum levels were measured by sandwich ELISA after 4 and 12 weeks (Leiden University Medical Center and University Medical Centre, Utrecht cohorts) or 4 and 16 weeks (Academic Medical Center/University of Amsterdam cohort).

Results. There was no difference in rituximab levels between progressors and non-progressors 4 weeks and 12 or 16 weeks after initiation of treatment in the different cohorts. There was also no correlation between rituximab levels at week 4 or week 12 or 16 and change in SHS score after 1 year.

Conclusion. Low rituximab serum levels are not associated with progression of structural damage in RA patients. The results do not support the use of dosages higher than 2\(\times\)1000 mg rituximab to inhibit progression of joint destruction.

Key words: rheumatoid arthritis, biologics, rituximab, B cells, radiology.

Introduction

Treatment with rituximab reduces disease activity in patients with RA. In parallel, rituximab is protective against progression of joint destruction in most patients, even in the absence of a clinical response [1–4]. However, a subset of patients still has progression of structural damage despite rituximab treatment [3, 5].

In the IMAGE trial, treatment with a relatively low dosage of 2\(\times\)500 mg rituximab in 2 weeks time induced a similar decrease in disease activity compared with the regular treatment with 2\(\times\)1000 mg rituximab. However, treatment with 2\(\times\)1000 mg rituximab induced more effective inhibition of joint destruction compared with 2\(\times\)500 mg rituximab in the first 24 weeks post-treatment [4]. These data suggest that rituximab may have a different dosage effect for inhibition of erosion formation and disease activity.

Previously we found that rituximab levels after administration of 2\(\times\)1000 mg rituximab differ markedly between RA patients [6]. Conceivably, low rituximab levels after 2\(\times\)1000 mg rituximab may be related to less effective inhibition of progressive joint damage. If confirmed, this
would strengthen the rationale for treatment with a high vs a low dosage of rituximab. Therefore we analysed whether low rituximab serum levels are associated with progression of structural damage in three cohorts of RA patients starting rituximab treatment.

**Patients and methods**

**Patients**

Patients were included from three independent prospective cohort studies of the response to rituximab treatment in RA that were reported previously [7–10]. All patients had active RA [DAS evaluated in 28 joints (DAS28) ≥3.2]. The study was approved by the Medical Ethics Committee of the Academic Medical Center, University of Amsterdam; all patients gave written informed consent obtained according to the Declaration of Helsinki.

**Study design**

Patients were treated with two infusions of 1000 mg of rituximab (days 1 and 15). Pre-medication with methylprednisolone was omitted in the Academic Medical Center/University of Amsterdam (AMC) cohort [9]. In all cohorts the DAS28 was obtained at baseline and after 24 weeks. Patients of the Leiden University Medical Center (LUMC) cohort were in all cases re-treated with rituximab after 6 months (fixed re-treatment), whereas patients of the AMC and University Medical Centre, Utrecht (UMCU) cohort were only re-treated when their DAS28 score was ≥3.2 at 6 months after initiation of treatment (on-demand re-treatment). We included patients in this analysis only if radiographs obtained before and 1 year after treatment were available and when serum was available for measurement of at least one post-treatment level of rituximab.

**Measurement of rituximab levels**

Rituximab levels, measured after 4, 12 and 24 weeks (LUMC and UMCU) or 4, 16 and 24 weeks (AMC), were determined by sandwich ELISA. In short, anti-rituximab antibodies were generated in rabbits by immunization with rituximab F(ab)2. After purification of IgG with protein A–sepharose, reactivity against human IgG was removed by passage over a sepharose–IVIG column. IVIG is a therapeutic i.v. IgG preparation prepared from more than 1000 blood donors. The antibodies that did not bind to the column were not reactive with serum IgG but strongly recognized rituximab. They were used for coating the ELISA plate and, after biotinylation, also as conjugate. The detection limit of the assay is 0.08 ng/ml. Because sera are tested at 1:10 dilution or higher, the detection limit in serum is 0.8 ng/ml.

**Radiographic assessments**

Radiographs of the hands and feet were obtained at baseline and 1 year after the initiation of rituximab treatment. The same observer, who was blinded to radiograph sequence, evaluated paired radiographs using the Sharp–van der Heijde scoring method (SHS), which consists of the joint space narrowing score and erosion score (range 0–448) [11]. Patients were divided into progressors vs non-progressors based on the change from baseline in SHS after 1 year using three different definitions of progression: an increase in SHS of ≥1 point, ≥3 points or ≥5 points.

**Statistical analysis**

Differences in rituximab levels between progressors and non-progressors were compared using the Mann–Whitney U test for nonparametric data. The Pearson’s correlation coefficient was used to assess correlations. All statistical analyses were performed with SPSS 17.0 software (SPSS, Chicago, IL, USA).

**Results**

**Patient characteristics**

Results on the radiographic progression and at least one post-treatment rituximab level were available for 24, 25 and 13 patients from the different centres. Baseline clinical characteristics and clinical response are shown in Table 1. The only notable initial difference between the three cohorts is concomitant MTX use, which was lower in the UMCU cohort. There was a trend towards lower rituximab levels in MTX users vs non-MTX users at week 4 [median (interquartile range) µg/ml: 126 (98.6–161) vs 186 (108.5–191)], week 12 or 16 [3.00 (1.12–7.55) vs 8.18 (3.23–19.4)] and at week 24 [0.36 (0.05–1.73) vs 0.58 (0.13–10.7)], although interquartile ranges are large and the differences are not statistically significant (week 4: P = 0.083; week 12/16: P = 0.131; week 24: P = 0.651). This difference might be explained by chance, consistent with previous literature [12].

**No difference in rituximab serum levels between progressors and non-progressors**

First, we pooled the data of the three cohorts. Fig. 1A shows the rituximab levels at 4, 12 or 16 and 24 weeks after the start of treatment for progressors and non-progressors using the three different cut-offs. Based on an increase in SHS of ≥1 point, ≥3 points or ≥5 points, we found 37 (60%), 33 (53%) and 23 (37%) progressors, respectively. We did not find any (trend towards a) difference in levels of rituximab between progressors and non-progressors at any time point (Fig. 1A). Using the cut-off of an increase in SHS of ≥3 points, the rituximab levels between progressors and non-progressors at week 4 were 126 (72–181), n = 29, and 128 (102–160), n = 27, respectively (P = 0.74). In addition, the pooled levels of week 12 of the two cohorts were 6.0 (3.1–18), n = 29, and 8.2 (3.3–16.5), n = 9, respectively (P = 0.68).

Next we compared the rituximab levels between progressors and non-progressors of the three cohorts separately using the cut-off of an increase in SHS of ≥3 points. On week 4 we did not find any differences in the separate cohorts. Of note, on week 12 the UMCU cohort showed significantly higher rituximab levels in the progressor group [8.7 (3.3–25.0), n = 8, vs 0.5 (0.04–5.4), n = 5; P = 0.04], but for the LUMC cohort the rituximab levels...
**Table 1** Baseline characteristics and clinical response of patients

<table>
<thead>
<tr>
<th></th>
<th>AMC (n = 24)</th>
<th>LUMC (n = 25)</th>
<th>UMCU (n = 13)</th>
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<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
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<tr>
<td>Female, n (%)</td>
<td>18 (75)</td>
<td>19 (76)</td>
<td>9 (69)</td>
</tr>
<tr>
<td>Age (years), median (range)</td>
<td>55 (22–68)</td>
<td>51 (33–75)</td>
<td>58 (41–84)</td>
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<tr>
<td><strong>Baseline disease status</strong></td>
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<tr>
<td>IgM-RF positive, n (%)</td>
<td>22 (92)</td>
<td>22 (88)</td>
<td>13 (100)</td>
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<tr>
<td>ACPA positive, n (%)</td>
<td>21 (88)</td>
<td>21 (84)</td>
<td>11 (85)</td>
</tr>
<tr>
<td>DAS28, mean (s.d.)</td>
<td>6.3 (1.0)</td>
<td>6.0 (1.2)</td>
<td>6.6 (0.9)</td>
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<tr>
<td>ESR (mm/h), median (range)</td>
<td>37 (4–66)</td>
<td>44 (5–134)</td>
<td>44 (21–124)</td>
</tr>
<tr>
<td>CRP (mg/l), median (range)</td>
<td>22 (2–112)</td>
<td>24 (2–114)</td>
<td>34 (7–117)</td>
</tr>
<tr>
<td>Disease duration (years), median (range)</td>
<td>12 (1–29)</td>
<td>12 (1–53)</td>
<td>14 (3–21)</td>
</tr>
<tr>
<td><strong>Total SHS, median (range)</strong></td>
<td>39 (0–247)</td>
<td>49 (8–245)</td>
<td>68 (3–159)</td>
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<tr>
<td><strong>Medication</strong></td>
<td></td>
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<tr>
<td>Concomitant MTX, n (%)</td>
<td>24 (100)</td>
<td>20 (80)</td>
<td>6 (46)</td>
</tr>
<tr>
<td>Concomitant oral prednisone, n (%)</td>
<td>18 (75)</td>
<td>14 (56)</td>
<td>10 (77)</td>
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<td><strong>Clinical response 24 weeks after course 1</strong></td>
<td></td>
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<tr>
<td>Delta DAS28, mean (s.d.)</td>
<td>−1.7 (1.7)</td>
<td>−1.7 (1.0)</td>
<td>−2.1 (1.4)</td>
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<tr>
<td>EULAR-good (%)</td>
<td>4 (17)</td>
<td>4 (17)</td>
<td>2 (15)</td>
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<tr>
<td>EULAR-moderate (%)</td>
<td>12 (50)</td>
<td>15 (63)</td>
<td>8 (62)</td>
</tr>
<tr>
<td>EULAR-non (%)</td>
<td>8 (33)</td>
<td>5 (21)</td>
<td>3 (23)</td>
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*aClinical response after 24 weeks not available for one patient in the LUMC cohort.

**Fig. 1** Relationship between rituximab serum levels and progression of structural damage.

(A) Rituximab levels of progressors and non-progressors using different cut-offs are not different at any time point. Data of the three cohorts are pooled. Values represent the median and range. (B) No correlation was observed between the change in SHS after 1 year and the rituximab levels at the different time points. The filled dots represent the three seronegative patients (both IgM RF and ACPA negative).
tended to be lower in the progressor group \([5.9 (2.4-15.7), \quad n=21, \quad vs \quad 16.5 (10.8-23.0), \quad n=4; \quad P=0.08]\). Finally, we did not observe any difference in serum rituximab levels between progressors and non-progressors at week 16 in the AMC cohort \([1.8 (0.3-22.7), \quad n=4, \quad and \quad 3.5 (1.3-8.0), \quad n=19, \quad respectively; \quad P=0.42]\).

No correlation between change in structural damage and rituximab serum levels

We also analysed the continuous data on the change in SHS score after 1 year. We did not find any significant correlation with the rituximab serum levels at different time points (Fig. 1B). In addition, the three seronegative patients were randomly distributed throughout the dot plot.

No difference in progression of structural damage between re-treated and non-re-treated patients

Because different re-treatment regimens were followed in the different cohorts (fixed re-treatment vs on-demand re-treatment), some patients received re-treatment with rituximab in the first year after initiation of treatment and some did not. However, we did not find a (trend towards a) difference in progression of structural damage between the re-treated \((n=45)\) and non-re-treated patients \((n=17; \quad P=0.26; \quad data \quad not \quad shown)\).

Discussion

The results of this prospective cohort study show that there is no relationship between rituximab serum levels measured 4 or 12 or 16 weeks after initiation of treatment and progression of structural damage, based on three independent cohorts of RA patients. The data do not support the continued use of higher dosages of rituximab to inhibit progression of joint destruction.

In a previous study that we performed on the relationship between rituximab serum levels on the one hand and B cell depletion and clinical response on the other, we observed that rituximab levels are highly variable between patients, but that low rituximab serum levels cannot explain the persistence of synovial B cells or clinical non-response [6]. The lowest rituximab levels were found in patients with anti-rituximab antibodies (ARAs) [6]. In the current study it was not possible to reliably compare progression of destruction between patients with and without ARAs, as the proportion of patients forming ARAs after the first treatment course was low (9%) and for only half of those patients were paired radiographs available. Nonetheless, the data show that low rituximab levels are not related to progression of structural damage, suggesting that treatment with higher dosages than \(2 \times 1000 \text{ mg} \) rituximab is unlikely to be more effective in protecting against progressive joint destruction. This contrasts with rituximab treatment in non-Hodgkin lymphoma, where low rituximab levels are related to decreased response to therapy [13, 14]. In contrast, it might perhaps even be possible to lower the dose of rituximab re-treatment after a starting dose of \(2 \times 1000 \text{ mg} \). Recently \(2 \times 1000 \text{ mg} \) and \(2 \times 500 \text{ mg} \) were compared side by side in early active RA patients. Only initial treatment with \(2 \times 1000 \text{ mg} \) rituximab resulted in statistically significant protection against progression of structural damage at 1 year, whereas \(2 \times 500 \text{ mg} \) and \(2 \times 1000 \text{ mg} \) resulted in comparable clinical efficacy. Exploratory analysis suggested that re-treatment with \(2 \times 500 \text{ mg} \) rituximab after 24 weeks might also be protective in terms of inhibition of structural damage [4]. Future research is needed to address this question and to study if this also holds true for patients with established, late-stage RA.

This study has some limitations. First, the study is relatively small. Second, the degree of progression that can be reliably detected above the measurement error of the SHS is best determined by the smallest detectable change (SDC), which is usually around 3 [15]. Therefore we chose the cut-off value of an increase in SHS of 3 points to study the rituximab levels in the three separate cohorts. We could not calculate the SDC for this study because the radiographs were scored by one observer and the SDC is calculated with repeated scores of two observers. Of note, the cut-off values of 1 and 5 points also did not show a difference in rituximab serum levels.

The only notable initial difference between the three cohorts is the concomitant MTX use, which was lower in the UMCU cohort. However, this did not influence the rituximab serum levels after treatment. Taken together, our data do not support the use of dosages higher than \(2 \times 1000 \text{ mg} \) rituximab in order to more effectively inhibit progression of joint destruction.

Rheumatology key messages

- Low rituximab levels are not related to progression of structural damage in RA patients.
- Dosages of rituximab higher than \(2 \times 1000 \text{ mg} \) are unlikely to inhibit progression of joint destruction more effectively in RA patients.

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