B cells and B cell directed therapies in rheumatoid arthritis: towards personalized medicine
Thurlings, R.M.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
CHAPTER 8

CLINICAL RESPONSE, PHARMACOKINETICS, DEVELOPMENT OF HUMAN ANTI-CHIMERIC ANTIBODIES, AND SYNOVIAL TISSUE RESPONSE TO RITUXIMAB TREATMENT IN PATIENTS WITH RHEUMATOID ARTHRITIS
**Authors:**

ROGIER M. THURLINGS, ONNO TENG, KOEN VOS, DANIELLE M. GERLAG, LUCIEN AARDEN, STEVEN O. STAPEL, JACOB M. VAN LAAR, PAUL P. TAK, GERRIT JAN WOLBINK.

**Affiliations:**

1. Division of Clinical Immunology and Rheumatology, Academic Medical Center/ University of Amsterdam, the Netherlands
2. Dept. of Rheumatology, Leiden University Medical Center (LUMC), Leiden, the Netherlands
3. Jan van Breemen Institute, Amsterdam, the Netherlands
4. Sanquin Research, Amsterdam, the Netherlands
5. Musculoskeletal Research Group, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom


---

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

**Abstract**

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

**Methods:** Fifty-eight RA patients 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 5 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 compared to 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.

**Introduction**

Rituximab is an effective therapy for rheumatoid arthritis (RA). Recent studies have shown that rituximab induces an incomplete B cell depletion in the synovial tissue of a subset of RA patients 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. 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 (ARA) or other factors influencing pharmacokinetics. Therefore, we analyzed the relationship between these parameters in a cohort of RA patients starting rituximab treatment. The data were confirmed in an independent cohort.
PATIENTS AND METHODS

PATIENTS. Patients were included from 2 studies on the synovial tissue response to rituximab in RA that were reported previously. Patients had active RA (Disease Activity Score evaluated in 28 joints (DAS28) > 3.2) despite methotrexate treatment. The study protocol was approved by the Ethics Committee of the participating centers; all patients gave written informed consent.

TREATMENT REGIMEN. Patients were treated with 2 infusions of 1000 mg rituximab (day 1 and 15). Pre-medication with methylprednisolone was omitted in the AMC cohort. 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. Patients were retreated after at least 24 weeks.

IMMUNOHISTOCHEMISTRY. Synovial biopsies were collected by arthroscopy in 17 patients of the LUMC cohort and 24 patients of the AMC cohort as described previously. In the AMC cohort frozen sections were stained with anti-CD19 (Becton Dickinson, San Jose, CA) 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; Serotech, Oxford, UK) to detect plasma cells. The immunohistochemical stainings were quantified using digital image analysis (AMC) or semiquantitative evaluation, respectively (LUMC). The relationship between CD20+ B cells and rituximab levels was only analyzed for baseline samples, since rituximab bound to CD20 might interfere with the detection of B cells using anti-CD20.

STATISTICAL ANALYSIS. Student’s 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 first assess the relationship 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).

MEASUREMENT OF RITUXIMAB LEVELS AND ARAS. Rituximab levels and ARAs were measured after 4, 12 and 24 weeks (LUMC) or 4, 16 and 24 weeks (AMC).

LEVELS OF RITUXIMAB VARIABILITY 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 (Figure 1A). ARAs were detectable in 2 patients who had received methylprednisolone and in 3 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 compared to ARA negative patients, when possible, for further analyses involving ARAs. Rituximab levels in ARA positive patients were lower compared to ARA negative patients, from already 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 [Figure 1B]).

Baseline ESR negatively predicted rituximab levels at week 4 in both patient cohorts (AMC cohort: r = -0.17, P = 0.018; LUMC cohort: r = -0.23, P = 0.007); in the AMC cohort

FIGURE 1

Rituximab levels were measured in two cohorts comprising a total of 38 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.
TABLE No.1

Patient characteristics and clinical response.

<table>
<thead>
<tr>
<th>DEMOGRAPHICS (N=58)</th>
<th>AMC (N=20)</th>
<th>LUMC (N=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, no. (%)</td>
<td>24 (80)</td>
<td>20 (71)</td>
</tr>
<tr>
<td>BASELINE DISEASE STATUS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM-RF positive, no. (%)</td>
<td>25 (83)</td>
<td>24 (86)</td>
</tr>
<tr>
<td>ACPA positive, no. (%)</td>
<td>25 (90)</td>
<td>23 (82)</td>
</tr>
<tr>
<td>DAS28, mean (± SD)</td>
<td>6.5 ± 1.1</td>
<td>6.0 ± 1.2</td>
</tr>
<tr>
<td>ESR, median (range) mm/hour</td>
<td>37 (4-86)</td>
<td>46 (5-139)</td>
</tr>
<tr>
<td>CRP, median (range) mg/dl</td>
<td>29 (1.9-112)</td>
<td>25 (2.0-114)</td>
</tr>
<tr>
<td>MEDICATION</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concomitant methotrexate, no. (%)</td>
<td>30 (100)</td>
<td>21 (75)</td>
</tr>
<tr>
<td>Concomitant leflunomide, no (%)</td>
<td>0 (0)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Corticosteroids, no. (%)</td>
<td>21 (70)</td>
<td>11 (39)</td>
</tr>
<tr>
<td>CLINICAL RESPONSE 24 WEEKS AFTER COURSE 1 (N=58)</td>
<td>AMC (N=20)</td>
<td>LUMC (N=28)</td>
</tr>
<tr>
<td>DAS28, mean (± SD)</td>
<td>5.0 ± 1.9</td>
<td>4.5 ± 1.2</td>
</tr>
<tr>
<td>EULAR good (%)</td>
<td>4 (13)</td>
<td>5 (18)</td>
</tr>
<tr>
<td>EULAR moderate (%)</td>
<td>15 (50)</td>
<td>17 (61)</td>
</tr>
<tr>
<td>EULAR none (%)</td>
<td>11 (37)</td>
<td>4 (14)</td>
</tr>
<tr>
<td>CLINICAL RESPONSE 24 WEEKS AFTER COURSE 2 (N=47)</td>
<td>AMC (N=22)</td>
<td>LUMC (N=25)</td>
</tr>
<tr>
<td>DAS28, mean (± SD)</td>
<td>4.5 ± 1.7</td>
<td>3.9 ± 1.1</td>
</tr>
<tr>
<td>EULAR good (%)</td>
<td>5 (23)</td>
<td>9 (36)</td>
</tr>
<tr>
<td>EULAR moderate (%)</td>
<td>10 (46)</td>
<td>14 (56)</td>
</tr>
<tr>
<td>EULAR none (%)</td>
<td>7 (32)</td>
<td>2 (8)</td>
</tr>
</tbody>
</table>

* 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.

TABLE No.2

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

<table>
<thead>
<tr>
<th>RTX LEVELS WK 4</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistence of CD22+ B cells at wk 4</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Persistence of CD19+ B cells at wk 4</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>Change CD22+ B cells wk 4-16</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>Change CD19+ B cells wk 4-16</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>Change CD138+ plasma cells wk 4-16</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>Change CD79a+ B/plasma cells wk 0-12</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>Change CD138+ plasma cells wk 0-12</td>
<td>0.71</td>
<td></td>
</tr>
</tbody>
</table>

ARAS

Change CD22+ B cells wk 0-16 | 0.24 |
Change CD19+ B cells wk 0-16 | 1.00 |
Change CD138+ plasma cells wk 0-16 | 0.76 |
Change CD79a+ B/plasma cells wk 0-12 | 0.10 |
Change CD138+ plasma cells wk 0-12 | 0.37 |

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. A Academic Medical Centre/University of Amsterdam; L Leiden University Medical Centre.

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. 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

similar trends were also found for baseline CRP and DAS28 (for CRP: r = -0.23, P = 0.006; r = 0.065; for DAS28: r = -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 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 methylprednisolon pre-medication.

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 analyzed 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
FIGURE No.2a

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 to those in patients without detectable synovial B cells at that time point (i.e. 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 (Table 2; Figure 2A). Similarly, the rituximab levels at week 4 did not predict whether synovial B cells persisted or decreased further after 16 weeks (Figure 2B). 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 week 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 compared to responders ([AMC] P = 0.81, P = 0.33 for week 4 and 16; [LUMC] P = 0.58, P = 0.11 for week 4 and 12). ARA positive patients experienced a similar decrease in DAS28 and EU-LAR response 24 weeks after the first and second treatment course compared to ARA negative patients (P = 0.87 and P = 0.32, for the response to course 1 and 2, respectively; Figure 2C,D).

FIGURE No.2b

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 at week 4 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.
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 therapy. Nevertheless, patients with ARAs or relatively low rituximab levels experience on average similar depletion of synovial B lineage cells and a similar clinical response compared to those 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 [4]. 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 in terms of clinical signs and symptoms and structural outcomes will become available. Moreover, there is a clear need for the identification of biomarkers that may help to further optimise rituximab treatment in individual 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 in terms of clinical signs and symptoms and structural outcomes will become available. Moreover, there is a clear need for the identification of biomarkers that may help to further optimise rituximab treatment in individual patients.

REFERENCES


(7) Teng YK, Levarht EW, Toes RE, Huizinga TW, van Laar JM. Residual inflammation after rituximab treatment is associated with sustained synovial plasma cell infiltration and enhanced B-cell repopulation. ANN RHEUM DIS 2008; 68:75-80. [EPUB AHEAD OF PRINT].


(11) van Gestel AM, Haagasma CI, van Riel PL. Validation of rheumatoid arthritis improvement criteria that include simplified joint counts. ARTHRITIS RHEUM 1998; 41:1845-1850.


ACKNOWLEDGMENTS

We would like to thank the Pharmacologist Marleen Kemper and the Research Technicians Els de Groote, Kim van Houten and Henk de Wijze.