B cells and B cell directed therapies in rheumatoid arthritis: towards personalized medicine
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B Cells and B Cell directed therapies in Rheumatoid Arthritis

CHAPTER 9

DISEASE ACTIVITY-GUIDED RITUXIMAB THERAPY IN RHEUMATOID ARTHRITIS: THE EFFECTS OF RETREATMENT IN INITIAL NON-RESPONDERS VERSUS INITIAL RESPONDERS
OBJECTIVE. To explore the efficacy of re-treatment with rituximab in patients with rheumatoid arthritis (RA) who were initial nonresponders to treatment, and to evaluate the effects of rituximab in RA when retreatment is given in a standardized way based on the Disease Activity Score in 28 joints (DAS28), according to the international consensus statement.

METHODS. Patients with RA who had a DAS28 of >3.2 received up to 3 courses of rituximab at intervals of at least 6 months, regardless of whether the patient had responded to the first course of treatment with rituximab.

RESULTS. Of the 30 patients with RA who were included in the study, 26 could be evaluated for the efficacy of treatment after 6 months. Eighteen patients qualified for re-treatment at 6 months, 6 patients were re-treated at a later time point because of a disease relapse, and 2 other patients were not re-treated because they had low disease activity. Seven of the 24 patients who qualified for re-treatment had not exhibited clinical improvement after the first treatment course. These patients typically did not respond to subsequent courses of rituximab. Of interest, in the 17 patients who had exhibited a clinical response to the first course of rituximab, the second and third treatment courses resulted in European League Against Rheumatism responses similar to those observed after the first course, and no major relapses occurred before re-treatment.

CONCLUSION. Rituximab re-treatment is not effective in patients who do not exhibit clinical improvement after the first treatment course, which is consistent with the notion that such patients represent a different pathogenetic subset of RA. Patients who initially responded to rituximab treatment experienced sustained benefit from DAS28-based systematic re-treatment according to the international consensus statement.

Introduction

Rituximab, a chimeric monoclonal antibody targeting CD20 expressed on B cells, is an effective and safe treatment of rheumatoid arthritis (RA). Currently, a course of rituximab treatment consists of 2 infusions administered during a 2-week period. According to a recent consensus statement, rituximab treatment should be repeated if patients experience a clinical response to the first treatment course and significant disease activity remains or recurs. Currently, there are no data on re-treatment of patients with RA who do not exhibit clinical improvement after the first course of rituximab.

In patients who experience a clinical response to rituximab, the number of synovial B cells and especially B cell–derived plasma cells decreases after rituximab treatment, which is consistent with the original hypothesis that rituximab treatment may break a self-perpetuating course of proliferation of self-reactive pathogenic B cell clones causing RA. Apparently, rituximab is not able to break this course of inflammation in patients whose RA is unresponsive to therapy. The reasons for the variable response are unknown, but suboptimal depletion of B cells and B cell–derived plasma cells may be associated with autoimmunity in the tissue of patients who do not experience a clinical response to the first course of rituximab. If this hypothesis were true, such patients theoretically might benefit from re-treatment, resulting in
more pronounced B cell depletion. Alternatively, patients without a clinical response to the first course of rituximab may represent a different pathogenic subset of the clinical syndrome termed RA. In this case, a clinical response to retreatment obviously cannot be expected in patients who did not respond to treatment initially.

The recently published international consensus statement on the use of B cell–targeted treatment with rituximab in patients with RA recommends repeating treatment if patients experienced a clinical response to a first treatment course, at least 6 months have passed, and significant disease activity persists or a disease flare occurs. This consensus statement is based on the experience in open-label extension studies of registration trials. In these studies, physicians were allowed to re-treat patients with active disease after 4 months or 6 months, at their own discretion; this approach introduced some variability. In these studies, clinical responses were maintained and perhaps slightly improved after each course, and major disease flares were prevented. It is important to study whether use of the currently advised standardized re-treatment schedule is able to maintain a clinical response and prevent major disease flares, for the following reasons. First, recurrent disease flares are invalidating for the patient. Second, disease flares exert a disproportionately large effect on radiographic progression. Third, the persistence of B cells, and in particular B cell– derived plasma cells, in the synovial tissue of some patients after rituximab treatment suggests that retreatment before disease flares may be required to improve the clinical response.

To determine whether an initial nonresponse to rituximab treatment predicts a lack of response to subsequent courses of treatment, and to evaluate the clinical effects of systematic, disease activity–guided re-treatment with rituximab in accordance with the international consensus statement, we re-treated patients with RA according to a standardized re-treatment schedule based on the Disease Activity Score in 28 joints (DAS28), independent of the clinical response to the first course. Patients whose DAS28 was ≥3.2 after at least 6 months were re-treated.

**PATIENTS AND METHODS**

**PATIENTS.** The study group comprised patients with a diagnosis of RA according to the American College of Rheumatology (formerly, the American Rheumatism Association) 1987 revised criteria for the classification of RA and in whom disease remained active despite methotrexate (MTX) treatment. Active disease was defined by the presence of ≥4 tender joints and ≥4 swollen joints (of 28 joints assessed), as well as at least 1 of the following criteria: erythrocyte sedimentation rate ≥28 mm/hour, serum C-reactive protein level ≥15 mg/liter, or morning stiffness lasting ≥45 minutes. Patients negative for both IgM rheumatoid factor (IgM-RF) and anti–citrullinated protein antibodies (ACPA) were excluded from the study.

All study patients were receiving treatment with MTX (5–30 mg/week) for at least 3 months, with stable dosages for 4 weeks prior to inclusion. Stable low-dose prednisone therapy (≤10 mg/day) and nonsteroidal anti-inflammatory drug (NSAID) treatment were allowed. Treatment with all other disease-modifying antirheumatic drugs (DMARDs) and biologic agents was withdrawn at least 4 weeks prior to study inclusion, with a washout period for leflunomide, etanercept, adalimumab, and infliximab treatment of >8 weeks prior to inclusion. No alteration of DMARD therapy was allowed during the study period. The study protocol was approved by the Medical Ethics Committee of the Academic Medical Center/University of Amsterdam, and all patients gave written informed consent.

**TREATMENT REGIMEN AND CLINICAL EVALUATION.** Patients were treated with 2 infusions of rituximab (1,000 mg on days 1 and 15) (Roche, Woerden, The Netherlands). Pretreatment included clemastine (2 mg intravenously) and acetylsalicylic acid (3 g orally). In contrast to routine clinical practice, premedication with methylprednisolone was not given during the first course, since the patients had also participated in a study on the effects of rituximab on biomarkers, and this could have introduced bias. After an interval of at least 6 months after the start of the first rituximab treatment course, patients whose DAS28 was ≥3.2 received re-treatment with a second course of rituximab. We allowed a maximum delay of 1–2 months between the decision to re-treat and the administration of rituximab, for logistic reasons. The DAS28 was determined at baseline and every month after treatment and subsequent re-treatment. A clinically significant decrease in disease activity was defined as a moderate or good response according to the European League Against Rheumatism (EULAR) criteria, as measured during at least 2 consecutive study visits. A relapse of disease activity was defined as an increase of ≥0.6 in the DAS28 from the lowest achieved value. Patients were followed up for up to 2 years.

**STATISTICAL ANALYSIS.** In addition to descriptive statistics, we used Student’s paired t-tests to evaluate the change in the DAS28 after treatment, because these data were normally distributed. Changes in serum immunoglobulin titers were evaluated using the nonparametric Wilcoxon signed rank test for paired data.
### TABLE No.1

Characteristics of the 30 patients*

<table>
<thead>
<tr>
<th>Demographics</th>
<th>(n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range) years</td>
<td>56 (22-75)</td>
</tr>
<tr>
<td>Female, no. (%)</td>
<td>24 (80)</td>
</tr>
</tbody>
</table>

**Disease status**

- Disease duration, median (range) years: 12 (1-50)
- Erosive disease, no. (%): 30 (100)
- Nodular disease, no. (%): 11 (37)
- IgM-RF, median (IQR) kU/liter: 62 (35-141)
- ACPA, median (IQR) kU/liter: 352 (126-1268)
- DAS28, mean (± SD): 6.5 (1.1)
- ESR, median (IQR) mm/hour: 37 (22-52)
- CRP, median (IQR) mg/l: 29 (12-64)

**Medication**

- No. of previous DMARDs, median (range): 4 (2-9)
- No. of previous biologic agents, median (range): 2 (0-4)
- Methotrexate dosage, median (range) mg/week: 15 (5-30)
- Corticosteroids, no. (%): 21 (70)
- Prednisone dosage, median (IQR) mg/day: 7.5 (5-10)

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* *IgM-RF = IgM rheumatoid factor; IQR = interquartile range; ACPA = anti–citrullinated protein antibody; DAS28 = Disease Activity Score in 28 joints; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; DMARDs = disease-modifying antirheumatic drugs.*

### FIGURE No.1

**FIGURE 1.** Treatment flow chart.

EULAR = European League Against Rheumatism.

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**CLINICAL AND DEMOGRAPHIC CHARACTERISTICS OF THE PATIENTS.**

The clinical and demographic characteristics of the 30 patients who were included in the study are shown in Table 1. All patients had active disease despite MTX treatment, with a mean DAS28 of 6.5. All patients were positive for IgM-RF and/or ACPAs. Twenty-five patients had previously been treated with ≥ 1 tumor necrosis factor (TNF) blocker. The reasons for withdrawal differed between patients and for each compound: in 5 patients, 1 or more TNF blockers were withdrawn because of side effects; in 17 patients, 1 or more TNF blockers were withdrawn because of inefficacy (primary inefficacy in 10 patients and secondary inefficacy in 7 patients); in 3 patients, the first TNF blocker was withdrawn because of side effects, and a second and/or third agent was withdrawn because of inefficacy. All patients were treated with MTX (range 5–30 mg), with the dosage remaining stable during followup. Twentyone patients received concomitant oral low-dose prednisone (range 5–10 mg/day). One patient was treated with oral prednisone at a dosage of 20 mg/day, because of persistent high disease activity 1–2 months after the second treatment course; 6 months after the start of treatment, the dosage was decreased.
to 12.5 mg/day. This patient was excluded from the efficacy analysis.

**TABLE No.2**

<table>
<thead>
<tr>
<th>Total no. of AEs † (n = 30)</th>
<th>359</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1 Δ</td>
<td>319</td>
</tr>
<tr>
<td>Grade 2</td>
<td>20</td>
</tr>
<tr>
<td>Grade 3</td>
<td>20</td>
</tr>
<tr>
<td>Grade 4</td>
<td>0</td>
</tr>
<tr>
<td>SUSARS ‡</td>
<td>5</td>
</tr>
<tr>
<td>- arterial embolism</td>
<td>1</td>
</tr>
<tr>
<td>- pulmonary embolism</td>
<td>1</td>
</tr>
<tr>
<td>- severe infusion reaction</td>
<td>2</td>
</tr>
<tr>
<td>- toxic hepatitis</td>
<td>1</td>
</tr>
<tr>
<td>No. of infections B</td>
<td>48</td>
</tr>
<tr>
<td>Infections per patient-year</td>
<td>0.9</td>
</tr>
<tr>
<td>No. of serious infections §</td>
<td>1</td>
</tr>
<tr>
<td>Urinary tract infections</td>
<td></td>
</tr>
<tr>
<td>- cystitis</td>
<td>7 *</td>
</tr>
<tr>
<td>- urosepsis</td>
<td>1</td>
</tr>
<tr>
<td>Respiratory infections</td>
<td></td>
</tr>
<tr>
<td>- sinusitis</td>
<td>1</td>
</tr>
<tr>
<td>- infectious bronchitis</td>
<td>8 ~</td>
</tr>
<tr>
<td>- pneumonia</td>
<td>3</td>
</tr>
<tr>
<td>Skin infections</td>
<td></td>
</tr>
<tr>
<td>- erysipelas</td>
<td>0</td>
</tr>
<tr>
<td>- cellulitis</td>
<td>2</td>
</tr>
<tr>
<td>- infection burnwound</td>
<td>2</td>
</tr>
<tr>
<td>Fever of unknown origin</td>
<td>2</td>
</tr>
<tr>
<td>Fungal infections</td>
<td></td>
</tr>
<tr>
<td>- cutaneous mycosis</td>
<td>3</td>
</tr>
<tr>
<td>- oral candidiasis</td>
<td>2</td>
</tr>
<tr>
<td>- vaginal candidiasis</td>
<td>1</td>
</tr>
<tr>
<td>Viral infections</td>
<td></td>
</tr>
<tr>
<td>- herpes zoster</td>
<td>1</td>
</tr>
<tr>
<td>- recurrence herpes labialis</td>
<td>2</td>
</tr>
</tbody>
</table>

Number of adverse events in the 30 patients*

* The total number of adverse events includes infusion reactions and does not include infections. The grades for adverse events were assigned based on the Common Toxicity Criteria. † Requiring antibiotic, antimycotic, or antiviral treatment. ‡ Treated with intravenous antibiotics. § Includes 3 cases of recurrent cystitis. ¶ Includes 3 cases of recurrent infectious bronchitis.

**FIGURE No.2**

**RESULTS OF RE-TREATMENT IN PATIENTS WHO DID NOT RESPOND TO INITIAL TREATMENT.**

Seven patients who did not fulfill the EULAR response criteria after their first treatment course were re-treated. One patient was excluded from efficiency analyses after the second treatment course, because this patient was treated with an intermediate dose of oral prednisolone due to persistent high disease activity. Of the other 6 initial nonresponders, none fulfilled the EULAR response criteria after the second treatment course (Figure 1). After the second course, 2 patients withdrew from the study because of the lack of a clinical response. A third treatment course in 4 of these patients resulted in a moderate EULAR response in only 1, while the other 3 patients did not respond.

**RESULTS OF SYSTEMATIC RE-TREATMENT IN INITIAL RESPONDERS.**

Seventeen patients who had responded to the first course of rituximab treatment (14 with a moderate EULAR response and 3 with a good EULAR response) received a second course of treatment (Figure 1). Eleven of the 17 initial respond-
ers qualified for a second course 6 months after the first course, because of a DAS28 of ≥ 3.2, and (due to logistic reasons) were re-treated at 7–8 months. Six patients experienced a relapse at a later time point (between 338 and 500 days), after which they received a second course (Figure 2). The DAS28 did not return to baseline levels in any of the patients who experienced a relapse (Figure 3). In the 17 initial responders, the second treatment course resulted, on average, in clinical improvement: 4 patients experienced a good EULAR response, 10 had a moderate EULAR response, and only 2 did not fulfill the EULAR response criteria. One patient experienced an infusion-related reaction at the time of re-treatment, after which rituximab was discontinued.

The 4 patients who fulfilled the EULAR criteria for a good response after the second course of treatment did not receive a third course, because of a long-lasting decrease in disease activity (DAS28 ≥ 3.2) after the second treatment course. The remaining 12 patients received a third treatment course, which also resulted in sustained clinical responses: 2 patients experienced a good EULAR response, 10 had a moderate EULAR response, and 10 patients had a moderate EULAR response.

SUSTAINED IMPROVEMENT AFTER RE-TREATMENT IN INITIAL RESPONDERS.

In order to evaluate changes in the DAS28 over time in patients treated according to a systematic re-treatment schedule, we determined the change in the DAS28 in the group of initial responders receiving re-treatment (n = 16). In this group of initial responders, the DAS28 decreased significantly after the first treatment course, from a mean ± SD of 6.5 ± 1.1 at baseline to 4.3 ± 1.5, 6 months after treatment (P < 0.001) (Figures 2 and 3). The DAS28 was significantly lower 6 months after the second treatment course compared with the value 6 months after the first treatment course (decreasing from 6.5 ± 1.1 at baseline to 4.3 ± 1.5, 6 months after the first treatment [as noted above], and from 5.0 ± 1.3 on the first day of the second treatment course to 3.8 ± 1.5, 6 months after the second treatment; P = 0.036) (Figure 3).

The 5 patients who had never received TNF blockade treatment (all of whom were responders to the first course of rituximab) were also analyzed separately. All 5 of these patients maintained their clinical response after re-treatment, similar to the whole group of initial responders. In 4 of these 5 patients, the DAS28 was lower 24 weeks after the second course compared with the score 24 weeks after the first course (data not shown).

SAFETY OF THE DAS28-GUIDED RE-TREATMENT SCHEDULE.

For the assessment of safety, all 30 patients participating in the study were analyzed (Table 2). The rate of infections requiring antibiotics was 0.9/patient-year during 2 years of followup. Infections consisted of urinary tract infections, respiratory tract infections, skin infections, and fungal and viral infections (Table 2). One patient was admitted to the hospital for intravenous antibiotic treatment of urosepsis. Two patients experienced recurrence of herpes labialis, and 1 patient had herpes zoster infection. There were no serious opportunistic infections or infections with Mycobacterium tuberculosis. In 4 patients, IgM levels were below the lower limit of normal after 6 months (n = 1), 1 year (n = 1), and 2 years (n = 2). In these patients, we observed oral candidiasis (n = 2), cutaneous mycoses (n = 1), and pneumonia (n = 1). Recurrent cystitis occurred in a patient after curretage and in a patient with a previous history of recurrent cystitis. In none of the patients did the IgG or IgA level decrease to below the lower limit of normal.

FIGURE No.3
DISCUSSION

This study is the first to show the clinical response to repeated rituximab treatment in patients with RA that was unresponsive to the first treatment course. In addition, we evaluated re-treatment of patients whose RA responded to a first course of rituximab treatment according to the international consensus statement, using a systematic disease activity–guided re-treatment schedule. Patients with active disease (defined as a DAS28 of ≥ 3.2) received re-treatment at least 6 months after the previous course. We observed that patients who did not respond to the first course of rituximab therapy generally did not respond to subsequent courses. Furthermore, we showed that treatment according to the disease activity–guided strategy is able to sustain the clinical response in initial responders to rituximab treatment and prevent major disease flares.

Systematic re-treatment was generally well tolerated, and there was no clear-cut safety signal compared with previous trials. The relatively high number of adverse events can be explained by the high frequency of comorbidities in our study population, consisting mainly of therapy-refractory RA in patients with high disease activity. We did not observe any opportunistic infections or tuberculosis, although 1 patient experienced a recurrence of herpes zoster shortly after commencing rituximab treatment.

Of importance, the lack of a response to rituximab treatment appears to be a constant feature in patients whose RA does not respond to the first treatment course. This observation is consistent with the notion that perhaps RA is not a single pathogenetic entity, but comprises different subsets leading to similar common final pathways. Disease mechanisms independent of B cells might be driving synovial inflammation in patients not responding to rituximab treatment. It is also conceivable that B cell proliferation and plasma cell formation may continue to occur despite treatment with anti-CD20 antibodies. Differential inflammatory expression of lymphocyte survival factors, such as B lymphocyte stimulator, APRIL, or interleukin-6, might be involved in the persistence of these cells. It has also been suggested that Fc-γ receptor polymorphisms could explain the lack of efficacy in nonresponders to rituximab treatment, but recent data on patients with malignant lymphoma do not support this hypothesis. An alternative explanation may be an effect of complement inhibitory proteins, such as CD55, which could render patients insensitive to rituximab treatment. The possible role of these factors in patients with RA remains to be elucidated.

Disease activity–guided re-treatment with rituximab according to the international consensus statement, at intervals of at least 6 months, was able to maintain the clinical response and prevent major disease relapses, although it should be noted that the majority of the patients experienced some increase in disease activity before re-treatment. Still, the baseline DAS28 was not reached in any of the patients. We cannot exclude the possibility that the minor increase in disease activity observed after 6 months could have been prevented by re-treatment within the 6-month interval. Ideally, in a treatment-to-target strategy, repeat treatment should be given at the time point at which a clinical response is expected and the target DAS28 is not achieved (i.e., after 4 months), but data on the safety of this approach are as yet unavailable.

Among patients whose RA initially responded to rituximab treatment, the clinical response tended to be more pronounced after the second course of treatment. This observation is consistent with the data obtained from the open-label extension studies of the registration trials. It fits the hypothesis that fixed re-treatment before disease flare may induce a further decrease in synovial inflammation by extending the period during which the level of B cell renewal is reduced and the level of proinflammatory survival signals is low. Similarly, in patients with chronic lymphocytic leukemia or indolent non-Hodgkin’s lymphoma, maintenance with (lowdose) rituximab after induction therapy is effective in enhancing the response rate and prolonging therapyfree survival.

In conclusion, RA patients whose disease initially fails to respond to rituximab are unlikely to exhibit a response to subsequent treatment courses. In patients who have an initial response to rituximab, disease activity–guided re-treatment with rituximab according to the current international consensus statement is effective in sustaining clinical response and preventing major disease flares.

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