B cells and B cell directed therapies in rheumatoid arthritis: towards personalized medicine
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Chapter 8

CLINICAL RESPONSE, PHARMACOKINETICS, DEVELOPMENT OF HUMAN ANTI-CD20 MONOCLONAL ANTIBODIES, AND SYNOVIAL TISSUE RESPONSE TO RITUXIMAB TREATMENT IN PATIENTS WITH RHEUMATOID ARTHRITIS
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 1,6. 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.

**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).

**IMMUNOHISTOCHEMISTRY.** Synovial biopsies were collected by arthroscopy in 17 patients of the LUMC cohort and 24 patients of the AMC cohort as described previously 1,6. In the AMC cohort frozen sections were stained with anti-CD19 (Becton Dickinson, San Jose, CA) and anti-CD22 (CLB-B-I; 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-cyttoplasmic CD20 (clone L26) to detect B cells, anti-human CD79a (clone JCD117, both from Dickinson, San Jose, CA) and anti-human CD138 (clone B-B4; Serotec, 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 1,6.

**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).

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**FIGURE No.1**

**Results**

**PATIENT CHARACTERISTICS.**

Clinical response to the first and second treatment course was available for in total 58 and 47 patients, respectively. Clinical characteristics and clinical response are shown in Table 1.

**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 (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, 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
similar trends were also found for baseline CRP and DAS28 (for CRP: $r^2 = -0.23$, $P = 0.006$; $r = 0.065$; for DAS28: $r = -0.13$, $P = 0.092$). 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 methylprednisolone 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
occurred, at the group level B cells did not further decrease.

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 EULAR 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.2a

FIGURE No.2b
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 therapy. Nevertheless, patients with ARAs or relatively low rituximab levels experience an 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, ARA and systemic inflammation is in line with earlier observations in patients treated with infliximab \(^{15}\). 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) even when patients form ARAs. These findings are in line with 2 dose-ranging studies that showed no statistically significant difference in ACR20, ACR50, or ACR70 response between patients treated with 2500 mg compared to those treated with 2x1000 mg rituximab \(^{16}\). It should be noted that the group of ARA positive patients was relatively small and that higher serum levels could perhaps result in a clinical response of longer duration. The present study was not designed to address this possibility, since all patients were re-treated after 24 weeks if the DAS28 ≥ 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 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


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