Effects of DMARDs on citrullinated peptide autoantibody levels in RA patients—A longitudinal analysis

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Effects of DMARDs on citrullinated peptide autoantibody levels in RA patients—A longitudinal analysis

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Abstract

Objective: To study whether stable treatment with DMARDs affects anti-CCP2 antibody levels in patients with rheumatoid arthritis.

Methods: In this longitudinal observational study 100 RA patients were followed for anti-CCP2 IgG antibody (U/L) and total IgG level (mg/dL) every 6 months for a total period of 2.5 years. All patients received stable DMARD treatment during this period. Five groups comprising each 20 patients were analyzed as follows: (1) methotrexate (MTX) alone, (2) tumor necrosis factor inhibitors (TNFi), (3) tocilizumab (TCZ), (4) rituximab (RTX), and (5) abatacept (ABA).

Results: Baseline demographic and disease-specific characteristics were comparable between the 5 groups. Anti-CCP2 antibody levels did not show significant changes in patients treated with MTX (mean ± SEM: −24.1 ± 8.1%), TNFi (−14.0 ± 11.1%) or TCZ (−24.3 ± 11.3%) between baseline and the 2.5 years follow-up. In contrast, anti-CCP2 antibody levels significantly decreased during treatment with RTX (−75.6 ± 4.4%) and ABA (−82.5 ± 3.7%). With respect to total IgG levels, no significant changes were observed with MTX (−1.6 ± 3.5%), TNFi (2.5 ± 3.4%), TCZ (−4.4 ± 3.1%), or ABA (−2.4 ± 3.8%) over 2.5 years. In contrast, total IgG levels significantly decreased during treatment with RTX (−22.0 ± 3.7%).

Conclusions: DMARDs targeting the adaptive immune response such as ABA and RTX significantly lowered anti-CCP2 IgG levels, while cytokine inhibitors and methotrexate had no significant effects on anti-CCP2 IgG levels. RTX is the only DMARD, which also lowers total IgG level.

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Introduction

Disease-modifying anti-rheumatic drugs (DMARDs) permit robust suppression of inflammation in patients with rheumatoid arthritis (RA) [1,2]. In consequence, structural damage related to inflammation is minimized upon DMARD therapy and functional state and quality of life are maintained. The principal aim of DMARD therapy is to minimize inflammation and to achieve remission of inflammation in RA. All treatment targets to date are exclusively formulated around the inflammatory aspect of RA with the aim to abrogate inflammation in the joints associated with the typical signs and symptoms of RA.

With better options to effectively treat RA, the immunological features of the disease attract growing attention. Especially, in times, when remission of inflammation becomes a feasible treatment target [3], the question arises whether also immunological remission is within reach. At the moment we have very limited data whether typical features of autoimmunity in RA such as anti-citrullinated protein antibodies (ACPA) are affected by DMARDs [4,5]. Longitudinal studies with serial measurements of ACPA are needed to address this question. Since ACPA have recently been shown to actively participate in the disease process of RA, there is growing interest to understand whether these antibodies are persistent or can also regress during therapy [6–10]. The observation that the persistence of ACPA determines the likelihood of RA patients to relapse of DMARD tapering further underlines the necessity to uncover whether ACPA can be affected by current DMARD treatment [11].

To study this question, 100 RA patients comprising all major currently available DMARD regimens were followed for more than 2 years in order to test the effects of DMARDs on the cyclic
citrullinated peptide antibody response. In addition, immunoglobulin levels were monitored during the same time period.

Methods

Patients and study design

The study was designed as prospective non-randomized observational study with the primary aim to analyze the effect of stable biological DMARD therapy on anti-cyclic citrullinated peptide 2 (CCP2) antibody levels over 2 years. All patients were recruited at the Department of Internal Medicine 3 of the University of Erlangen-Nuremberg. To be included patients had to have (i) the diagnosis of RA according to the ACR-EULAR 2010 criteria, (ii) positive CCP2 antibody testing, and (iii) active disease with a DAS28 score of more than 3.2 units despite treatment with methotrexate (MTX) at sufficient dose (20 mg/week) and length (3 months; MTX-IR setting). Consecutive patients were enrolled starting with a new DMARD regimen with tumor necrosis factor inhibitor (TNFi), tocilizumab (TOC), rituximab (RTX), or abatacept (ABA) at standard doses. We aimed to recruit a total number of 80 patients (N = 20 per treatment modality) receiving the respective DMARD treatments in stable fashion over 2.5 years. In addition, a control group received only MTX was also enrolled with the aim of 20 patients in this control arm. In total 181 patients had to be included to achieve 100 patients, which had stable DMARD treatment over 2.5 years. 80 (4 times 20) of them with biological DMARDs and 20 with MTX. Only patients with at least 2.5 years of stable DMARD treatment were analyzed. In the residual 81 patients treatment with the respective DMARD had to be stopped because of lack of efficacy or intolerance, thus not allowing long-term follow-up analysis of anti-CCP2 autoantibody levels.

Serum analyses

Serum was analyzed in the same laboratory at day of clinical presentation throughout the observation period. CCP2 antibody levels were measured by enzyme linked immunosorbent assay from Phadia/Thermofisher Scientific. Levels for immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) levels were measured by nephelometry (Beckman Coulter). Antibody and immunoglobulins levels were measured at each time point.

Statistics

Group comparisons were performed by ANOVA if values were normally distributed. To compare dichotomous variables Kruskal Wallis test was used. P < 0.05 was considered significant.

Results

Patients' characteristics

The Table summarizes patients' characteristics. Briefly, 68% (68/100) were females, mean (± SD) age was 52.2 ± 13.2 years and mean (± SD) body mass index was 25.5 ± 4.8 units, all of them balanced between the 5 treatment groups. Patient had established RA with disease duration of 7.8 ± 5.3 years and active disease with a mean (± SD) of 4.7 ± 1.2 DAS28 units at baseline. Disease duration and activity was not different among the 5 groups. At 2.5 years follow-up mean DAS28 scores decreased to 2.6 ± 1.0 with no significant differences between the 5 groups. Baseline CCP2 antibody levels were balanced between the 5 groups with tendencies to higher levels in the ABA and RTX groups. Furthermore, total IgG, IgA, and IgM levels were virtually equal in the 5 groups and also the analysis of comorbidities did not show major differences.

Effect of DMARDs on anti-CCP2 antibody levels in individual RA patients

Analysis of anti-CCP2 antibody levels was performed every 6 months for a total period of 2.5 years during which patients received stable treatment with the respective DMARD. Based on the large differences in anti-CCP2 antibody levels at baseline we normalized the baseline value (=100%) and reported relative changes to baseline (Fig. 1A–E). Analysis of individual patients showed consistent decreases in anti-CCP2 antibody levels only in patients treated with RTX and ABA, while patients receiving MTX, TNFi, or TCZ did not show any clear reduction in the levels. Furthermore, when analyzing absolute levels by plotting anti-CCP2 antibody levels in a logarithmic scale, similar observations were made with decreases in the levels in RTX and ABA treatment (Fig. 1F–J).

Effects of different DMARDs on anti-CCP2 antibody levels

Anti-CCP2 antibody levels did only show a minor and non-significant decrease in the group of patients receiving MTX over the entire observation period of 2.5 years. Mean (± SEM) decreases

<table>
<thead>
<tr>
<th>Table</th>
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<tr>
<td></td>
<td>MTX</td>
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<tr>
<td>BMI (kg/m²)</td>
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<td>Dis. duration (y)</td>
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<td>CCP2 titer (U/L)</td>
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<td>IgG level (mg/dL)</td>
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<td>IgA level (mg/dL)</td>
<td>305 ± 117</td>
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<tr>
<td>IgM level (mg/dL)</td>
<td>141 ± 73</td>
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*ABA, abatacept; BMI, body mass index; Dis. duration, disease duration; CCP, anti-cyclic citrullinated peptide 2 autoantibodies; CHD, concomitant cardiovascular disease; COPD, concomitant chronic obstructive pulmonary disease; DAS28 BL, disease activity score 28 at baseline; DAS28 FU, disease activity score 28 at last follow-up; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; MTX, methotrexate; N, number; RTX, rituximab; TNFi, tumor necrosis factor alpha inhibition; TCZ, tocilizumab. P values refer to group comparisons by ANOVA in case of parametric variable or Kruskal Wallis test in case of nonparametric variables.*
Fig. 1. Effects of DMARDs on CCP2 autoantibody titers. Effects of (A and F) methotrexate (MTX), (B and G) tumor necrosis factor alpha inhibitors (TNFi), (C and H) tocilizumab (TCZ), (D and I) rituximab (RTX), and (E and J) abatacept (ABA) are shown. Each line represents single patient. Left graphs indicate relative changes to baseline level (− 100%) (A–E) and right graphs in logarithmic scale indicate absolute levels (B–J).
of anti-CCP2 antibody levels were 24.1 ± 8.1% in the MTX, 14.0 ± 11.1% in the TNFi, and 24.3 ± 11.3% in the TCZ group (all non-significant) (Fig. 2A). In contrast, anti-CCP2 antibody levels significantly decreased during treatment with RTX showing a 64.0 ± 7.2% reduction after 1 year and a 75% reduction after 2.5 years. Similarly, antibody levels significantly decreased after ABA treatment with a 58.0 ± 6.0% reduction after 1 year and an 82.5 ± 3.7% reduction after 2.5 years. Seroconversion was observed in 5 patients treated with ABA, 2 patients treated with RTX, and a single patient treated with TCZ. In non-responders, no significant changes of anti-CCP2 antibody levels were observed, although there was a tendency toward lower levels in RTX- and ABA-treated patients as well (Fig. 2B). When comparing, responders (DAS28 remission/low disease activity) with non-responders, however, we observed a significantly stronger decrease in anti-CCP2 antibody levels in responders to ABA and RTX (Fig. 2C and D).

Figure 2. Effects of DMARDs on CCP2 autoantibody levels and immunoglobulin levels. (A and B) Percent change of anti-citrullinated peptide 2 (CCP2) antibody levels in the 5 treatment groups in responders (A) and non-responders (B). (C and D) Comparison of anti-CCP2 antibody levels in responders and non-responders receiving ABA or RTX. Baseline level is set 100%. MTX, methotrexate; TNFi, tumor necrosis factor alpha inhibitors; TCZ, tocilizumab; RTX, rituximab; ABA, abatacept.

Effects of different DMARDs on immunoglobulin levels

Total IgG level remained stable during the entire observation period in patients treated with MTX, TNFi, and TCZ (Fig. 3A). While no changes of IgG level was observed with ABA, they decreased during RTX treatment and were significantly lower than in the other treatment groups (741 ± 41 mg/dL; −22.0 ± 3.7%; ANOVA P < 0.0001). No changes in IgA levels were recorded in the different groups (Fig. 3B) and IgM level only decreased in the RTX group (72 ± 11 mg/dL; ANOVA P < 0.0001), which was in accordance with the observations made for IgG (Fig. 3C). Finally, when plotting anti-CCP2 antibody and IgG changes in the individual patient, a random distribution was observed for patients receiving MTX, TNFi, and TCZ, whereas ABA and RTX were shifted to lower anti-CCP2 antibody levels and only RTX shifted to lower IgG levels (Fig. 3D).
Discussion

In this longitudinal study we show differential effects of established DMARD treatments on anti-CCP2 autoantibody levels in patients with RA. We analyzed patients who started a new DMARD regimen and maintained it over at least 2 years. The clinical response in such patients was excellent with all patients achieving low disease activity and most of them remission. However, the individual DMARD regimen varied substantially in their effects on anti-CCP2 autoantibody levels. While CCP2 autoantibody levels remained virtually unchanged in patients receiving continuous MTX, TNFi or TCZ treatment, levels significantly decreased with the co-stimulation inhibitor abatacept or the B cell depleting antibody rituximab.

These observations suggest that anti-CCP2 autoantibody responses may be subdued in some RA patients, particularly if DMARDs interfering with the adaptive immune response are used. RTX globally depletes B cells and therefore blocks differentiation of plasmablasts and plasma cells leading to lowering of total immunoglobulin production [12]. This concept is supported by the observation that aside from anti-CCP2 autoantibodies also total IgG level decreased during treatment with RTX. Decreased total IgG has been associated with an increased infection risk in patients receiving RTX, and is therefore of concern [13]. Notably, decrease of anti-CCP2 autoantibody with ABA occurs without concomitant decrease in total IgG. Abatacept interferes with the immunological synapse and inhibits T cell-mediated B cell stimulation [14]. In particular, abatacept, but not TNFi interferes with T follicular helper cell differentiation and phosphorylation of Syk, which represent essential mechanisms required for B cell differentiation into plasma cells in the lymphoid follicle [15]. Inhibitory effects of ABA on anti-CCP2 autoantibody levels and even seroconversion have also been described in the preliminary analysis of anti-CCP2 autoantibody levels in a recent study with ABA [16]. In contrast to a recent study showing significant decreases of anti-CCP2 autoantibody levels in RA patients treated with TNFi [17] we were not able to confirm such significant effects albeit a trend toward a decrease in autoantibody levels were observed. Nonetheless, our head-to-head study suggests that the effects of RTX and ABA on anti-CCP2 autoantibody levels are more pronounced than the ones observed with TNFi.

While control of inflammation remains the primary target of DMARD treatment in RA, it is tempting to speculate that aside from clinical remission also immunological remission may be achievable. Abrogation of the immune response against citrullinated proteins would allow moving closer to immunological remission. The growing knowledge that anti-CCP2 autoantibodies actively participate in the course of RA supports this concept. Several studies have shown that these autoantibodies enhance osteoclast formation and bone destruction in patients with RA [6–8]. In addition, pulmonary changes in RA patients have been associated with anti-CCP2 autoantibodies [9,10]. Furthermore, continuous presence of CCP2 antibodies is associated with recurrence of RA, when DMARDs are tapered, suggesting that anti-CCP2 autoantibodies are essentially in the onset and maintenance of inflammation in RA [11].

It needs to be mentioned, however, that the value of reducing autoantibodies in RA patients is currently unknown. Clinical response and even remission does not necessarily require reduction of autoantibodies, since our data from MTX-, TNFi-, and TCZ-treated patients nicely show that remission can be reached in the absence of significant decreases of autoantibody levels. Furthermore, TNFi and TCZ have shown to allow excellent protection of bone in RA patients despite continuous presence of autoantibodies. This effect can most likely be explained by the effective inhibition of osteoclast differentiation by TNF and IL-6R blockade.
which both act downstream of autoantibodies. Nonetheless, some positive effects of reduction of antibodies and even seroconversion can be still envisioned: DMARD tapering in patients in remission, for instance, has shown to be more successful in the absence of underlying autoimmunity. Hence, sustained clinical remission combined with seroconversion (immunological remission) could emerge as treatment goal, which facilitates the successful tapering and even stopping of DMARD treatment.

Taken together these data show that anti-CCP2 autoantibodies decrease upon treatment with DMARDs interfering with adaptive immune response. A limitation of this study is the samples size. While we were able to show significant differences when following one hundred patients, larger studies are needed in the future to assess the effects of different biological DMARD regimens on anti-CCP2 autoantibodies. In summary, our data suggest that autoimmunity against citrullinated proteins may be reversible in principle, bringing immunological remission of RA within reach.

Key messages
1. Significant decrease of anti-CCP2 autoantibody levels in rheumatoid arthritis patients receiving stable treatment with abatacept or rituximab.
2. Significant decrease of serum immunoglobulin G and M levels in rheumatoid arthritis patients receiving stable treatment with rituximab.
3. No effect of tumor necrosis factor inhibitors, tocilizumab, or methotrexate on anti-CCP2 autoantibody and immunoglobulin G and M levels.

Acknowledgments
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References