The value of rheumatoid factor and anti-citrullinated protein antibodies as predictors of response to infliximab in rheumatoid arthritis: an exploratory study

Ruth Klaasen, Tineke Cantaert, Carla A. Wijbrandts, Christine Teitsma, Danielle M. Gerlag, Theo A. Out, Monique J. de Nooijer, Dominique Baeten and Paul P. Tak

Abstract

Objective. It remains unclear whether autoantibodies are useful biomarkers to tailor the choice of biological treatment in RA. We investigated the relationship between the presence and levels of different RF and ACPA isotypes and the response to TNF blockade in an exploratory study.

Methods. A total of 101 active RA patients were prospectively treated with infliximab (3 mg/kg). Changes in disease activity were monitored by the 28-joint DAS (DAS-28). Serum levels of different isotypes [immunoglobulins M, G and A (IgM, IgG and IgA)] of RF and anti-citrullinated peptide antibodies were measured by ELISA.

Results. The mean DAS-28 decreased from 5.9 (1.1) at baseline to 4.0 (1.3) at Week 16 of infliximab treatment (P < 0.001). High baseline levels of different isotypes of RF (all P < 0.008), ACPA IgM (P = 0.008) and ACPA IgG (P = 0.07) were associated with an absolute decrease in DAS-28 after TNF blockade. This relationship persisted after adjusting for DAS-28 at baseline. However, the different isotypes of baseline RF and ACPA levels accounted for only a small proportion of variance in treatment response (RF: R^2 between 7 and 12% and ACPA: R^2 between 4 and 7%). The simultaneous presence of all three isotypes of RF or ACPA had no additive value.

Conclusion. Presence as well as the titres of RF and IgM ACPA at baseline are significantly correlated with better response to infliximab treatment. However, this correlation is not strong enough to allow a reliable prediction in individual patients.

Trial Registration. ISRCTN Register, http://www.controlled-trials.com/isrctn/, ISRCTN36847425.

Key words: Rheumatoid arthritis, Autoantibodies, Response, Biologicals, Biomarker, Tumour necrosis factor, Rheumatoid factor, ACPA, Infliximab, Isotypes.

Introduction

Increasing understanding of the cellular and molecular pathophysiology of RA has enabled the rapid development of innovative biological agents that target specific parts of the immune response [1]. Several of these targeted therapies were demonstrated to have a major impact on inflammation as well as structural damage in clinical trials as well as daily practice and thereby broadened and improved the treatment options in RA. However, the response to these different biological treatments is heterogeneous between RA patients [2, 3] and the use of these agents is associated with significant risk of adverse events and considerable cost [4, 5]. Therefore, the prediction of individual response to biological treatment has become a major clinical challenge in RA.

RA is nowadays thought to be a clinical syndrome comprising different pathogenic subsets [6, 7]. Growing...
evidence suggests that the presence of ACPA defines a specific RA subset. Indeed, specific gene–environment interactions involving the HLA-DR4 shared epitope are mainly found in ACPA+ RA, at least in North-Western Europe [6, 8], and clonal alterations of synovial T cells are elevated in ACPA+ vs ACPA- patients [9]. Additionally, treatment responses to B-cell depletion with the anti-CD20 antibody rituximab are superior in ACPA and/or RF-positive RA vs seronegative RA [10, 11]. In contrast to B-cell depletion, several studies have suggested that high levels of RF and/or ACPA correlate with a decreased response to TNF blockade [12–14]. Taken together, these data suggest that differences in cellular and molecular mechanisms of disease in seropositive vs seronegative RA may have clinical implications for tailoring the choice of targeted therapy.

Upon critical review of the published literature, however, it appears that the relationship between RF or ACPA and response to TNF blockade is far from unequivocal. In contrast to the previously mentioned reports [12–14], several studies found no difference [15, 16] or even an increase in clinical response [17] to TNF blockade [12].

The baseline demographic and clinical features of the larger cohort have been described previously by Wijbrandts et al. [20] and are summarized in Table 1 for those who were included in the present study. Patients were selected here based on the availability of serum at baseline combined with standardized follow-up data on the response to infliximab treatment.

Use of oral CSs (≤10 mg/day) and NSAIDs was allowed if the dose had not been changed within 1 month before baseline. IA steroid injections within the last month before inclusion and prior use of biological treatment were not allowed. All patients were subsequently treated by i.v. infusions with infliximab in a dosage of 3 mg/kg at baseline, Weeks 2 and 6 and subsequently every 8 weeks. Patients depicting a reduction of the DAS-28 of at least 1.2 (twice the measurement error of the DAS-28 over time) at Week 16 of infliximab treatment were defined as responders, representing a significant clinical improvement [21]. In 77 of the patients, sera were also available at Week 16, which allowed us to investigate the effect of infliximab treatment on the autoantibody levels. All patients gave written informed consent according to the Declaration of Helsinki and the study was approved by the Medical Ethics Committee of the AMC/University of Amsterdam.

### Autoantibody analysis

Immunoglobulin M (IgM), immunoglobulin G (IgG) and immunoglobulin A (IgA) RFs were measured by ELISA (IgM RF ELISA; Sanquin, Amsterdam, The Netherlands; IgA RF and IgG RF ELISA: NO.VA Qanta Lite, San Diego, CA, USA). Positivity for all RFs was defined as ≥20 U/ml. ACPA IgG was measured by the anti-CCP2 ELISA kit (Immunoscan RA, Mark2, Eurodiagnostica NO.RA-96RT, Arnhem, The Netherlands) according to the manufacturer’s instructions. Values ≥25 U/ml were considered positive. IgA and IgM ACPA were measured with the anti-CCP2 ELISA kit after substitution of the secondary antibody with a goat anti-human IgM (Novus, Cambridge, UK) or a goat anti-human IgA (Caltbiochem Merck, Schiphol-Rijk, The Netherlands). Horseradish

### Methods

#### Patients and samples

Sera were collected from 101 patients fulfilling the ACR classification criteria for RA [18] and having active disease defined by a DAS evaluated in 28 joints (DAS-28) [19] ≥3.2 despite the use of MTX (5–30 mg/week). ESR was used to calculate the DAS-28.

<table>
<thead>
<tr>
<th>Table 1 Baseline patient characteristics in the total cohort, in responders (decrease in DAS-28 ≥ 1.2 after 16 weeks) and non-responders</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients (n = 101)</td>
</tr>
<tr>
<td>Age, mean (s.d.), years</td>
</tr>
<tr>
<td>Gender: female, n (%)</td>
</tr>
<tr>
<td>Previous DMARDs, mean (s.d.)</td>
</tr>
<tr>
<td>Disease duration, median (IQR), months</td>
</tr>
<tr>
<td>ESR, median (IQR)</td>
</tr>
<tr>
<td>CRP, median (IQR)</td>
</tr>
<tr>
<td>DAS-28, mean (s.d.)</td>
</tr>
<tr>
<td>Erosive disease, n (%)</td>
</tr>
<tr>
<td>MTX dosage, mean (s.d.), mg/week</td>
</tr>
<tr>
<td>Prednisone use, n (%)</td>
</tr>
</tbody>
</table>

Baseline characteristics were compared between responders and non-responders.
peroxidase-labelled antibody was diluted 1:3000 for IgA ACPA and 1:10 000 for IgM ACPA (high performance ELISA buffer; Sanquin, The Netherlands). For this modified ELISA, values are expressed as arbitrary units/millilitre (AU/ml). Cut-off values for IgA and IgM ACPA positivity were defined as the mean plus 2 s.d. of the values obtained in a group of 45 healthy controls who did not have a diagnosis of RA or other rheumatic disease, similar to previously defined cut-off points [22, 23]. This definition resulted in cut-off values for positivity of 1.25 AU/ml for IgA anti-CCP2 and of 1.6 AU/ml for IgM anti-CCP2.

Statistical analysis
Continuous data were described as mean (s.d.) if normally distributed and as median, inter quartile range (IQR) if not normally distributed. The unpaired t-test or, where appropriate, Mann–Whitney U-test (MWU test) was used to compare responders and non-responders. The Pearson’s and, where appropriate, Spearman’s correlation test was used to investigate the relationship between autoantibody levels and disease activity. Categorical data were represented as percentages (%) and were analysed using the Chi-squared or Fisher’s exact test. Cox regression analysis and multiregression analysis were used to investigate the predictive value of baseline autoantibody levels, using log transformation of RF and ACPA levels to fit in the linear regression analysis. Finally, the Wilcoxon signed rank test was used to compare autoantibody levels over time. All statistical analyses were performed with SPSS 16.0 for Windows (SPSS, Chicago, IL, USA).

Results
Baseline clinical and serological characteristics and response to treatment
We first aimed to assess whether the cohort of patients used for the present biomarker study was comparable with the previously described large patient cohorts starting TNF blockers. As shown in Table 1, age, gender, disease duration and previous DMARD use were representative of prototypical RA cohorts. As to the serology, 62% were positive for IgM RF and 76% were positive for IgG ACPA at baseline. All patients used MTX (mean dosage of 19 mg/week) and 23% were on prednisone. Despite this treatment, the patients had active disease as evidenced by a DAS-28 score of 5.9 (1.1) [mean (s.d.)]. Following infliximab treatment, DAS-28 score decreased to 4.0 (1.3) [mean (s.d.)] at Week 16 (P < 0.001). Sixty-nine (68%) of the 101 patients experienced a decrease in DAS-28 > 1.2 and were thus classified as responders. Taken together, these data indicate that the baseline clinical and demographic features, the response to treatment and the serological profile of this cohort are comparable with what has been described for other large cohorts of active RA patients eligible to TNF blockade, and thus indicate that this cohort is appropriate and representative to explore the value of autoantibodies as predictive biomarker for response to treatment with infliximab [13, 23, 24].

The clinical response to infliximab treatment is not correlated with the baseline immunoglobulin levels
Before analysing whether autoantibody levels at baseline predict response to treatment, we aimed to exclude potential biases related to the global Ig levels. Total serum levels of IgM, IgA and IgG were not different between responders and non-responders (Table 2) and were not related to the degree of response in a continuous analysis (data not shown).

The clinical response to infliximab treatment is correlated with the baseline RF levels
In this cohort of 101 established RA patients, 63 (62%) were positive for IgM RF, 33 (33%) for IgA RF and 24 (24%) for IgG RF at baseline (Table 2). The presence of IgM RF at baseline was more frequent in the responder vs non-responder group (P = 0.009), with similar numerical trends that did not reach statistical significance for the other RF isotypes (Table 2, Fig. 1). However, even for IgM RF the variance of response was small (R² = 0.065). Accordingly, the positive predictive value (PPV) of the presence of IgM RF for identifying responders to infliximab treatment was 78% and the negative predictive value (NPV) was 46%.

As not only the presence but also the levels of autoantibodies may determine treatment response, we next used a continuous rather than dichotomous approach to analyse the data. The decrease in DAS-28 was significantly correlated with the baseline levels of all three RF isotypes (all RF r between 0.224 and 0.306 and P < 0.025). Also after adjustment for baseline DAS-28 levels in a linear multi-regression model, the baseline titres of all three RF isotypes predicted response to infliximab treatment. Despite this significant association, however, the baseline RF levels accounted for only a small proportion of variance in treatment responses (IgM RF: R² = 0.109, P = 0.001; IgG RF: R² = 0.117, P = 0.001; IgA RF: R² = 0.068, P = 0.008). Accordingly, modification of the cut-off levels for the different RF isotypes did not result in a marked improvement of the PPV and NPV for response to treatment, as illustrated in Table 2.

Finally, we assessed whether the presence of several RF isotypes improved the prediction compared with the use of single isotypes. Sixteen (23%) out of 69 responders were positive at baseline for all 3 RF isotypes in contrast to 3 (9%) out of 32 non-responders (P = 0.10) (Table 2). The presence of all three isotypes reached a PPV of 84% and a NPV of 32%, which was only slightly higher when compared with the PPV or NPV of the single isotypes (Table 2). Also when response was defined according to the European League Against Rheumatism (EULAR) response criteria, the results were similar; these data are shown in a supplementary table, available as supplementary data at Rheumatology Online.

Taken together, these data indicate that the presence as well as the titres of RF at baseline is significantly
Table 2: Percentage of presence and level of all different isotypes of RF and ACPA at baseline in responders (decrease in DAS-28 ≥ 1.2) vs non-responders as well as PPV and NPV for the prediction of clinical response to infliximab treatment in RA.

<table>
<thead>
<tr>
<th>Isotypes of RF/ACPA</th>
<th>All patients</th>
<th>Responders</th>
<th>Non-responders</th>
<th>PPV, %</th>
<th>NPV, %</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total IgM, mean (s.d.), g/l</td>
<td>1.6 (0.8)</td>
<td>1.5 (0.7)</td>
<td>1.8 (0.9)</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total IgG, mean (s.d.), g/l</td>
<td>11.7 (3.6)</td>
<td>11.9 (3.7)</td>
<td>11.0 (3.4)</td>
<td>0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total IgA, mean (s.d.), g/l</td>
<td>3.3 (1.1)</td>
<td>3.3 (1.1)</td>
<td>3.2 (1.0)</td>
<td>0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM RF+, n (%)</td>
<td>63 (62)</td>
<td>49 (71)</td>
<td>14 (44)</td>
<td>78</td>
<td>46</td>
<td><strong>0.009</strong></td>
</tr>
<tr>
<td>IgM RF+ level median (IQR), U/ml</td>
<td>34 (10–133)</td>
<td>43 (15–169)</td>
<td>15 (2–53)</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG RF+, n (%)</td>
<td>24 (24)</td>
<td>20 (36)</td>
<td>4 (13)</td>
<td>83</td>
<td>37</td>
<td>0.07</td>
</tr>
<tr>
<td>IgG RF+ level, median (IQR), U/ml</td>
<td>5 (3–17)</td>
<td>3 (1–21)</td>
<td>2 (1–8)</td>
<td><strong>0.02</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA RF+, n (%)</td>
<td>33 (33)</td>
<td>25 (36)</td>
<td>8 (25)</td>
<td>76</td>
<td>34</td>
<td>0.26</td>
</tr>
<tr>
<td>IgA RF+ level, median (IQR), U/ml</td>
<td>7 (2–28)</td>
<td>10 (2–40)</td>
<td>5 (1–20)</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All isotypes RF+, n (%)</td>
<td>19 (19)</td>
<td>16 (23)</td>
<td>3 (9)</td>
<td>84</td>
<td>32</td>
<td>0.10</td>
</tr>
<tr>
<td>IgM RF &gt; 100 μ/ml, n (%)</td>
<td>28 (28)</td>
<td>19 (27)</td>
<td>9 (27)</td>
<td>100</td>
<td>32</td>
<td>0.02</td>
</tr>
<tr>
<td>IgG RF &gt; 100 μ/ml, n (%)</td>
<td>4 (4)</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>100</td>
<td>32</td>
<td>0.17</td>
</tr>
<tr>
<td>IgA RF &gt; 100 μ/ml, n (%)</td>
<td>6 (6)</td>
<td>4 (6)</td>
<td>2 (2)</td>
<td>100</td>
<td>32</td>
<td>0.09</td>
</tr>
<tr>
<td>IgG-CCP2+, n (%)</td>
<td>77 (76)</td>
<td>56 (81)</td>
<td>21 (66)</td>
<td>73</td>
<td>44</td>
<td>0.09</td>
</tr>
<tr>
<td>IgG-CCP2 level, median (IQR), U/ml</td>
<td>244 (28–966)</td>
<td>361 (62–1033)</td>
<td>120 (38–200)</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGA-CCP2+, n (%)</td>
<td>52 (51)</td>
<td>38 (55)</td>
<td>14 (44)</td>
<td>54</td>
<td>58</td>
<td>0.29</td>
</tr>
<tr>
<td>IgA-CCP2 level, median (IQR), AU/ml</td>
<td>1.7 (0.5–4.9)</td>
<td>1.8 (0.5–6.5)</td>
<td>1.1 (0.4–2.6)</td>
<td>0.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGM-CCP2+, n (%)</td>
<td>63 (62)</td>
<td>49 (71)</td>
<td>14 (44)</td>
<td>83</td>
<td>44</td>
<td><strong>0.01</strong></td>
</tr>
<tr>
<td>IgM-CCP2 level, AU/ml</td>
<td>1.6 (0.9–4.8)</td>
<td>1.9 (0.9–5.4)</td>
<td>1.1 (0.7–2.5)</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All CCP2+ isotypes, n (%)</td>
<td>42 (42)</td>
<td>37 (47)</td>
<td>5 (23)</td>
<td>79</td>
<td>39</td>
<td>0.06</td>
</tr>
</tbody>
</table>

CCP2: anti-citrullinated peptide 2. Bold values have a P-value (responders vs non-responders) of <0.05.

Fig. 1: Baseline levels (T=0) and after 16 weeks of infliximab treatment (T=1) of the different isotypes of RF and ACPA in responders and non-responders according to the absolute decrease in DAS-28. Median with IQR (Wilcoxon signed rank test). *P < 0.05 and **P < 0.001.
correlated with better response to infliximab treatment. However, this correlation is not strong enough to allow a reliable prediction in individual patients, even when combining different RF isotypes.

Clinical response to infliximab treatment is correlated with baseline IgG and IgM ACPA levels

In the same cohort of 101 established RA patients, 77 (76%) were positive for IgG ACPA, 52 (51%) were positive for IgA ACPA and 50 (50%) were positive for IgM ACPA (Table 2). Using a dichotomous analysis, the presence of IgM ACPA was significantly higher in responders vs non-responders ($P=0.003$), with a similar trend for IgG (Table 2, Fig. 1). As for RF, however, the variance of response was small ($R^2=0.027$). Accordingly, the PPV was 80% and the NPV was 40%. No difference in IgA ACPA was found between responders and non-responders (Table 2, Fig. 1). Continuous analysis revealed that levels of IgM and IgG ACPA, but not IgA ACPA, correlated with the decrease in DAS-28 score during 16 weeks of infliximab treatment (IgM ACPA: $r=0.264$ and $P=0.008$; IgG ACPA: $r=0.181$, $P=0.07$). After adjusting for DAS-28 at baseline (before start of infliximab treatment) in a multiregression model, these two isotypes of ACPA remained independent predictors of response. However, the variance of response was even smaller for the RF (IgM: ACPA $R^2=0.069$, $P=0.008$ and IgG RF: $R^2=0.043$, $P=0.037$). In a supplementary table (available as supplementary data at Rheumatology Online), level and presence of the three isotypes of ACPA in relationship to the EULAR response are shown; the results are comparable to those when clinical improvement is defined by a reduction of the DAS-28 of at least 1.2. From these data we conclude that the presence and titres of IgM and IgG ACPA at baseline are related to a better clinical response to infliximab treatment, but that this association is not strong enough for prediction of response in individual patients.

Both RF and ACPA levels tend to decrease during infliximab treatment in responding RA patients

Previous studies did not always measure autoantibodies at baseline, but also used values measured during TNF blockade in a proportion of patients. Therefore, we tested whether TNF blockade modulates autoantibody titres and may thus induce biases in serological analyses.

In the total cohort all isotypes of RF decreased (all $P<0.002$). The median level of IgM RF decreased by 13% (40–35 U/ml), IgG RF by 17% (6–5 U/ml) and IgA RF by 25% (8–6 U/ml). Also IgG ACPA decreased by 20% (419–336 U/ml) ($P=0.036$). IgA ACPA tended to decrease by 19% (1.98–1.61 AU/ml) ($P=0.07$), but IgM ACPA was not decreased after treatment (1.90–2.08) ($P=0.26$).

In responders the decrease in RF was more pronounced (IgM RF, IgG RF and IgA RF decreased by, respectively, 20, 22 and 35%, all $P<0.002$) than for the whole cohort (Fig. 1). Respectively 14% of IgM RF, 41% of IgG RF and 11% of IgA RF-positive responders transformed to seronegative IgM, IgG or IgA RF status. In non-responders, levels of IgM, IgG and IgA RF increased numerically by, respectively, 44, 67 and 20%, although none of these changes reached statistical significance (Fig. 1). In the responding patients, IgG and IgA ACPA levels were also decreased [respectively, 20% ($P=0.041$) and 19% ($P=0.015$)]. Respectively, 4% of IgG ACPA and 3% of IgA ACPA-positive responders transformed to seronegative IgG and IgA ACPA status.

A weak correlation was found between the decrease in DAS-28 and the decrease in the levels of IgG RF ($r=0.291$ and $P=0.012$) and IgM RF ($r=0.189$, $P=0.10$) after 16 weeks of treatment. No correlation was found between the decrease in DAS-28 after 16 weeks and the reduction in IgA RF or the different ACPA isotypes. In conclusion, in responders all isotypes of RF and IgG and IgA ACPA decreased and this was highly significant for RF.

Discussion

This study prospectively examined the value of different isotypes of RF and ACPA as predictors of response to infliximab in a representative cohort of established RA patients. Presence and levels of different isotypes of RF and IgM and IgG ACPA were related to clinical response to infliximab at group level but this association is not strong enough to predict response in individual patients. The combination of the presence of different autoantibodies or isotypes had no additive value in predicting response to infliximab treatment in RA patients.

Our results showing that the presence and levels of RF and/or ACPA are related to a better response to TNF blockade appear to be at first sight in contrast to other studies. Two studies showed that high levels of RF [13] or the presence of RF [14] were related to a decreased clinical response to TNF blockade, whereas two other studies did not find any correlation between RF status and response [12, 16]. As for IgG ACPA, reports indicated either no correlation [13, 15], a negative correlation [12, 14] or a positive correlation between autoantibody status and the response to TNF blockade [17]. There may be several reasons explaining these differences. First, all these studies assessed clinical response at 6 months [12,14–16] or even up to 1 year [13]. At these later time points, clinical parameters may not reflect primary response to treatment but also secondary loss of response, which can be influenced by totally unrelated mechanisms like the development of antibodies to infliximab [25] or adalimumab [26]. Secondly, 10% of patients had discontinued TNF blockade due to inefficacy and had started alternative treatments before response was measured at 6 months in one of these studies, which may have biased the results [14]. Thirdly, two studies did not determine the autoantibody status at baseline but at various time points during anti-TNF treatment [14, 16]. As previously demonstrated and confirmed in the present study, TNF blockade as such can modulate RF and, to a lesser extent ACPA, which may have biased the data of these two studies [12, 13, 15, 23, 27]. During treatment, we found that levels of all isotypes of RF and
IgG/IgA ACPA decreased by ~20% and 20–35%, respectively, which is in accordance with other studies [12, 13, 15, 27]. Collectively, these considerations emphasize the importance of a stringent, prospective study design to assess reliably the value of candidate biomarkers.

Although we found statistically significant positive correlations between autoantibody levels and response to TNF blockade, the explained variance of response was relatively small (~7–11%) for the different RF isotypes and even less for the different ACPA isotypes. Consistent with the clinical experience that the response to TNF blockade is not a dichotomous phenomenon [28], there was no distinct threshold value for RF or ACPA to predict reliably the clinical response to treatment. Combining different isotypes of RF or ACPA or using higher cut-off levels of RF did not further contribute to prediction of response. Hence, RF and ACPA isotype levels are statistically associated with response to treatment, but cannot be translated into a predictive test in individual patients.

Taken together, the results presented here support the notion that there may be differences in treatment response between autoantibody-positive patients compared with those who are autoantibody negative. RF and ACPA, however, cannot be used in isolation to predict the response to anti-TNF antibody treatment in the context of personalized medicine.

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### Supplementary data

Supplementary data are available at *Rheumatology* Online.

### References


