Rheumatoid arthritis: predictors of clinical response to TNF blockade
Klaasen, R.

Citation for published version (APA):
CHAPTER 2

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

Ruth Klaasen¹, Tineke Cantaert¹, Carla A. Wijbrandts¹, Christine Teitsma¹, Danielle M. Gerlag¹, Theo A Out², Monique J de Nooijer², Dominique Baeten¹, and Paul P. Tak¹

¹Division of Clinical Immunology and Rheumatology and ²Division of Experimental Immunology, Academic Medical Center/University of Amsterdam, Amsterdam, the Netherlands

*Rheumatology (Oxford) 2011 Aug;50(8):1487-93
ABSTRACT

Objective: It remains unclear whether auto-antibodies are useful biomarkers to tailor the choice of biological treatment in rheumatoid arthritis (RA). We investigated the relationship between the presence and levels of different rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA) isotypes and the response to tumor necrosis factor (TNF) blockade in an exploratory study.

Methods: 101 active RA patients were prospectively treated with infliximab (3mg/kg). Changes in disease activity were monitored by the disease activity score (DAS28). Serum levels of different isotypes (IgM, IgG, IgA) of RF and anti-citrullinated peptide antibodies were measured by enzyme-linked immunosorbent assay.

Results: The mean DAS28 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 DAS28 after TNF blockade. This relationship persisted after adjusting for DAS28 at baseline. However, the different isotypes of baseline RF and ACPA levels accounted for only a small proportion of variance in treatment responses (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 titers 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.
Increasing understanding of the cellular and molecular pathophysiology of rheumatoid arthritis (RA) has enabled the rapid development of innovative biological agents that target specific parts of the immune response. 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 and the use of these agents is associated with significant risk of adverse events and considerable cost. 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. Growing evidence suggests that the presence of anti-citrullinated protein antibodies (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, and clonal alterations of synovial T cells are elevated in ACPA+ versus ACPA- patients. Additionally, treatment responses to B cell depletion with the anti-CD20 antibody rituximab are superior in ACPA and/or rheumatoid factor (RF) positive RA versus seronegative RA. In contrast to B cell depletion, several studies have suggested that high levels of RF and/or ACPA correlate with a decreased response to tumour necrosis factor (TNF) blockade. Taken together, these data suggest that differences in cellular and molecular mechanisms of disease in seropositive versus 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 with the previously mentioned reports, several studies found no difference or even an increase in clinical response to TNF blockade in seropositive versus seronegative diseases. Thus, it remains uncertain whether autoantibodies are useful biomarkers to tailor the choice of TNF blockade versus other targeted therapies such as B cell depletion in RA. Therefore, we conducted an exploratory study in a large cohort of active RA patients to investigate the exact relationship between the presence and levels of RF and ACPA (including their different isotypes) and the response to TNF blockade by infliximab.

METHODS
Patients and samples
Sera were collected from 101 patients fulfilling the American College of Rheumatology (ACR) classification criteria for RA and having active disease defined by a disease activity score evaluated in 28 joints (DAS28) ≥ 3.2 despite the use of methotrexate (5-30 mg/week). Erythrocyte sedimentation rate (ESR) was used to calculate the DAS28.

The baseline demographic and clinical features of the larger cohort have been described previously by Wijbrandts et al 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 corticosteroids (≤ 10 mg/day) and non-steroidal anti-inflammatory drugs (NSAIDs) was allowed if the dose had not been changed within one month prior to baseline. Intra-articular steroid injections within the last month before inclusion and the prior use of...
biological treatment were not allowed. All patients were subsequently treated by intravenous 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 DAS28 of at least 1.2 (twice the measurement error of the DAS28 over time) at week 16 of the infliximab treatment were defined as responders, representing a significant clinical improvement. 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
IgM, IgG and IgA RF were measured by enzyme-linked immunosorbent assay (ELISA) (IgM RF ELISA: Sanquin, Amsterdam, The Netherlands; IgA RF and IgG RF ELISA: INOVA Qanta Lite, San Diego, CA). Positivity for all RF was defined as ≥ 20 U/ml. ACPA IgG was measured by the anti-CCP2 ELISA kit (ImmunoScan RA, Mark2, Euro Diagnostica NO.RA-96RT, Arnhem, the Netherlands) according to the manufacturer’s instructions. Values of ≥ 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, United Kingdom) or a goat anti-human IgA (Calbiochem Merck, Schiphol-Rijk, Netherlands). The HRP labeled antibody was diluted 1:3000 for IgA ACPA and 1:10000 for the IgM ACPA (HPE buffer, Sanquin, The Netherlands). For this modified ELISA, values are expressed as arbitrary units/ml (AU/ml). Cut off values for IgA and IgM ACPA positivity were defined as the mean plus 2 standard deviations (SD) 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. This definition resulted in cut off values for positivity of 1.25 AU/ml for the IgA anti-CCP2 and of 1.6 AU/ml for the IgM anti-CCP2.

Statistical analysis
Continuous data were described as mean ± SD if normally distributed and as median, inter quartile range (IQR) if not normally distributed. The unpaired t-test or, where appropriate, Mann Whitney U (MWU) test was used to compare responders and non-responders. The Pearson 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 analyzed using the Chi-square or Fishers’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).

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 to previously described large patient cohorts starting TNF blockers. As shown in Table 1, age, gender, disease duration, and previous disease modifying anti-rheumatic drug
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 methotrexate (mean dosage of 19 mg/week) and 23% were on prednisone. Despite this treatment, the patients had active disease as evidenced by a DAS28 score of 5.9 ± 1.1 (mean ± SD). Following infliximab treatment DAS28 score decreased to 4.0 ± 1.3 (mean ± SD) at week 16 (P < 0.001). Sixty-nine of the 101 patients (68%) experienced a decrease in DAS28 ≥ 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 to what has been described for other large cohorts of active RA patients eligible to TNF blockade and thus that this cohort is appropriate and representative to explore the value of auto-antibodies as predictive biomarker for response to treatment with infliximab13,23,24.

The clinical response to infliximab treatment is not correlated with the baseline immunoglobulin levels

Before analyzing whether auto-antibody 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 versus non-responder group (P = 0.009), with similar numerical trends that did not reach statistical significance for the other RF isotypes (Table 2, Figure 1). However, even for IgM RF the variance of response was small (R² = 0.065). Accordingly, the

| Table 1. Baseline patient characteristics in the total cohort, in responders (decrease in DAS28 ≥ 1.2 after 16 weeks), and non-responders. Data are represented as mean ± SD, median (IQR) or numbers, % as appropriate. Baseline characteristics were compared between responders and non-responders. DMARD = disease modifying anti-rheumatic drugs ESR = erythrocyte sedimentation rate CRP = C-reactive protein DAS28 = disease activity score measured in 28 joints MTX = methotrexate. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | All patients (n=101) | Responders (n=69) | Non-responders (n=32) | P-value |
| Age (years)     | 55 ± 13          | 54 ± 13          | 57 ± 13          | 0.29           |
| Female          | 75, 74%          | 54, 78%          | 21, 66%          | 0.38           |
| Previous DMARDs | 2.1 ± 1.6        | 2.0 ± 1.5        | 2.3 ± 1.7        | 0.31           |
| Disease duration (months) | 79 (44-159)    | 76 (36-149)    | 98 (45-185)    | 0.34           |
| ESR             | 28 (14-41)       | 31 (20-41)       | 25 (11-41)       | 0.09           |
| CRP             | 11 (4-24)        | 12 (4-25)        | 8 (4-28)         | 0.37           |
| DAS28           | 5.9 ± 1.1        | 6.0 ± 1.0        | 5.6 ± 1.2        | 0.12           |
| Erosive disease | 77, 77%          | 54, 78%          | 23, 72%          | 0.48           |
| MTX dosage (mg/week) | 19 ± 8            | 20 ± 8            | 17 ± 9            | 0.14           |
| Prednisone use  | 23, 23%          | 18, 26%          | 5, 16%           | 0.24           |
The decrease in DAS28 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 DAS28 levels in a linear multi-regression model, the baseline titers of all 3 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: $R^2 = 0.109$, $P = 0.001$; IgG RF: $R^2 = 0.117$, $P = 0.001$; IgA RF: $R^2 = 0.068$, $P = 0.008$). Accordingly, modification of the cut-off levels for the different RF isotypes did not result in a marked improvement of the positive and negative predictive value for response to treatment, as illustrated in Table 2.

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

Taken together, these data indicate that the presence as well as the titers of RF 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, even when combining different RF isotypes.

The clinical response to infliximab treatment is correlated with the 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 versus non-responders (P = 0.003), with a similar trend for IgG (Table 2, Figure 1). As for RF, however, the variance of response was small (R² = 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, Figure 1). Continuous analysis revealed that levels of IgM and IgG ACPA, but not IgA ACPA, correlated with the decrease in DAS28 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 DAS28 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 than for the RF (IgM: ACPA R² = 0.069, P = 0.008 and IgG RF: R² = 0.043, P = 0.037).

Figure 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 DAS28. Median with IQR (Wilcoxon signed rank Test). (* P < 0.05 and ** P < 0.001).
In a supplementary table, 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 DAS28 of at least 1.2. From these data we conclude that the presence and titers 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 auto-antibodies 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 to 35 U/ml), IgG RF by 17% (6 to 5 U/ml) and IgA RF by 25% (8 to 6 U/ml). Also IgG ACPA decreased by 20% (419 to 336 U/ml) \( (p = 0.036) \). IgA ACPA tended to decrease by 19% (1.98 to 1.61 AU/ml) \( (p = 0.07) \), but IgM ACPA was not decreased after treatment (1.90 to 2.08) \( (p = 0.26) \).

In responders the decrease in all 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 (Figure 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 (Figure 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 DAS28 and the decrease in the levels of IgG RF \( (r = 0.291 \text{ and } p = 0.012) \) and IgM RF \( (r = 0.189, p = 0.10) \) after 16 weeks of treatment. No correlation was found between the decrease in DAS28 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 the group level but this association is not strong enough to predict response in individual patients. The combination of the presence of different auto-antibodies 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 with other studies. Two studies showed that high levels of RF\(^1\) or the presence of RF\(^4\) were related to a decreased clinical response to TNF blockade, whereas two other studies did not find any correlation between RF
status and response\textsuperscript{12,16}. As for IgG ACPA, reports indicated either no correlation\textsuperscript{13,15}, a negative correlation\textsuperscript{12,14}, or a positive correlation between the autoantibody status and the response to TNF blockade\textsuperscript{17}. There may be several reasons explaining these differences. Firstly, all these studies assessed clinical response at 6 months\textsuperscript{12,14-16} or even up to one year\textsuperscript{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 against infliximab\textsuperscript{25} or adalimumab\textsuperscript{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\textsuperscript{14}. Thirdly, two studies did not determine the autoantibody status at baseline but at various time points during anti-TNF treatment\textsuperscript{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\textsuperscript{12,13,15,23,27}. During treatment we found that levels of all isotypes of RF and IgG/ IgA ACPA decreased by approximately 20% respectively 20%-35% which is in accordance with other studies\textsuperscript{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 auto-antibody levels and response to TNF blockade, the explained variance of response was relatively small (about 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\textsuperscript{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 to those who are autoantibody negative. RF and ACPA can however not be used in isolation to predict the response to anti-TNF antibody treatment in the context of personalized medicine.

**ACKNOWLEDGEMENTS**

We wish to thank the research nurses Nitolanda van Rijn and Natasja Cassin for performing clinical assessments and also Desiree Pots and Andrea J.W. Hoevenberg for performing the ELISA of the different auto-antibodies, and Eurodiagnostica (Arnhem, The Netherlands) for donating part of the reagents.

**FUNDING**

This study was funded by a Health Care Efficiency Research Program grant from The Netherlands Organization for Health Research and Development (ZonMw) in assignment of The Netherlands Organization for Scientific Research (NWO) (grant number 945-02-029), the Dutch Arthritis Association and the European Community’s FP6 funding (Autocure) and the Center for Translational Molecular Medicine (TRACER). This publication reflects only the authors' views;
the European Community is not liable for any use that may be made of the information herein.

Dominique Baeten is supported by a Vidi grant of The Netherlands Organization for Scientific Research.

REFERENCE LIST


ACPA, RF AND RESPONSE TO INFliximab

Supplemental table. Percentage of presence and level of all different isotypes of RF and anti-citrullinated protein antibodies (ACPA) at baseline in EULAR good/moderate responders versus EULAR non-responders as well as positive predictive value (PPV) and negative predictive value (NPV) for the prediction of clinical response to infliximab treatment in RA. Data are represented as mean ± SD, median (IQR) or percentages, % as appropriate.

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>EULAR response</th>
<th>EULAR non –response</th>
<th>PPV</th>
<th>NPV</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total IgM (g/l)</td>
<td>1.6 ± 0.8</td>
<td>1.6 ± 0.7</td>
<td>1.7 ± 0.9</td>
<td>0.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total IgG (g/l)</td>
<td>11.7 ± 3.7</td>
<td>11.8 ± 3.6</td>
<td>11 ± 3.9</td>
<td>0.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total IgA (g/l)</td>
<td>1.8 ± 0.9</td>
<td>3.3 ± 1.1</td>
<td>3.1 ± 1.1</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM RF+ (%)</td>
<td>63, 62%</td>
<td>54, 68%</td>
<td>9, 41%</td>
<td>86%</td>
<td>34%</td>
<td>0.02</td>
</tr>
<tr>
<td>IgM RF, level (U/ml)</td>
<td>34 (10-166)</td>
<td>40 (14-166)</td>
<td>8 (2-52)</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG RF+ (%)</td>
<td>24, 24%</td>
<td>22,28%</td>
<td>2,90%</td>
<td>92%</td>
<td>26%</td>
<td>0.07</td>
</tr>
<tr>
<td>IgG, RF, level (U/ml)</td>
<td>5 (3-17)</td>
<td>6 (3-22)</td>
<td>3 (2-5)</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA RF+ (%)</td>
<td>33, 33%</td>
<td>28, 35%</td>
<td>5, 23%</td>
<td>85%</td>
<td>25%</td>
<td>0.26</td>
</tr>
<tr>
<td>IgA, RF, level (U/ml)</td>
<td>7 (2-28)</td>
<td>10 (2-39)</td>
<td>3 (1-15)</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All isotypes RF+ (%)</td>
<td>19, 19%</td>
<td>17, 22%</td>
<td>2, 1%</td>
<td>89%</td>
<td>24%</td>
<td>0.19</td>
</tr>
<tr>
<td>IgM RF &gt;100 u/ml</td>
<td>28, 28%</td>
<td>26, 33%</td>
<td>2, 1%</td>
<td>93%</td>
<td>27%</td>
<td>0.03</td>
</tr>
<tr>
<td>IgG RF &gt; 100 u/ml</td>
<td>4, 4%</td>
<td>4, 5%</td>
<td>0, 0%</td>
<td>100%</td>
<td>23%</td>
<td>0.28</td>
</tr>
<tr>
<td>IgA RF &gt;100 u/ml</td>
<td>6, 6%</td>
<td>6, 8%</td>
<td>0, 0%</td>
<td>100%</td>
<td>23%</td>
<td>0.18</td>
</tr>
<tr>
<td>IgG- CCP2+(%)</td>
<td>77, 76%</td>
<td>65, 82%</td>
<td>12, 55%</td>
<td>84%</td>
<td>42%</td>
<td>0.01</td>
</tr>
<tr>
<td>IgG-CCP2, level (U/ml)</td>
<td>244 (28-966)</td>
<td>345 (65-985)</td>
<td>41 (2-875)</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGA-CCP2+(%)</td>
<td>52, 51%</td>
<td>44, 56%</td>
<td>8, 36%</td>
<td>85%</td>
<td>29%</td>
<td>0.11</td>
</tr>
<tr>
<td>IGA-CCP2, level (AU/ml)</td>
<td>1.7 (0.5-4.9)</td>
<td>1.8 (0.6-5.8)</td>
<td>0.7 (0.3-2.0)</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGM-CCP2+(%)</td>
<td>50, 50%</td>
<td>44, 56%</td>
<td>6, 27%</td>
<td>88%</td>
<td>31%</td>
<td>0.02</td>
</tr>
<tr>
<td>IGM-CCP2, level (AU/ml)</td>
<td>1.6 (0.9-4.8)</td>
<td>1.7 (1.0-5.4)</td>
<td>1.0 (0.6-2.1)</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All isotypes ACPA+(%)</td>
<td>41, 41%</td>
<td>37, 47%</td>
<td>5, 23%</td>
<td>88%</td>
<td>29%</td>
<td>0.04</td>
</tr>
</tbody>
</table>


