Rheumatoid factor, but not anti-cyclic citrullinated peptide antibodies, is modulated by infliximab treatment in rheumatoid arthritis

L De Rycke, X Verhelst, E Kruithof, F Van den Bosch, I E A Hoffman, E M Veys, F De Keyser


Although the precise aetiology of rheumatoid arthritis (RA) remains elusive, evidence for autoimmunity is strong because several autoantibodies are associated with the disease. Besides the rheumatoid factor (RF), another group of autoantibodies was detected in the serum of patients with RA: the anti-cyclic citrullinated peptide (anti-CCP) antibodies. Recently, we compared the diagnostic value of the RF and anti-CCP antibodies in a consecutive cohort of patients with inflammatory joint symptoms (patients with RF and anti-CCP antibodies in a consecutive cohort of patients with RA: the anti-cyclic citrullinated peptide (anti-CCP) antibodies in a consecutive cohort of patients with RA: the anti-cyclic citrullinated peptide (anti-CCP) antibodies. The differential effect of infliximab treatment on IgM RF and anti-CCP antibodies, and the different predictive value on changes in acute phase reactants during infliximab treatment support the existing evidence that RF and anti-CCP antibodies are independent autoantibody systems in RA.

Objectives: To analyse the effect of infliximab on IgM rheumatoid factor (RF) and anti-cyclic citrullinated peptide (CCP) antibodies, and determine whether baseline autoantibody titres (IgM RF and anti-CCP antibodies) are associated with changes in acute phase reactants.

Patients and methods: 62 patients with refractory RA were treated with infliximab combined with methotrexate. At baseline and week 30, serum samples were tested for IgM RF by two agglutination assays, and for anti-CCP antibodies by an ELISA. Percentage change in C reactive protein (CRP) and erythrocyte sedimentation rate (ESR) was calculated.

Results: At baseline and week 30 RF titres were reduced significantly during infliximab treatment (p<0.001 and p=0.038, respectively), whereas anti-CCP antibodies were unchanged (p=0.240). Baseline IgM RF titres, but not anti-CCP antibodies, correlated inversely with changes in CRP and ESR during treatment. Patients with a marked decrease in acute phase reactants had lower IgM RF titres than those with a smaller decrease in CRP and ESR; no significant differences were found for anti-CCP antibodies.

Conclusion: The differential effect of infliximab treatment on IgM RF and anti-CCP antibodies, and the different predictive value on changes in acute phase reactants during infliximab treatment support the existing evidence that RF and anti-CCP antibodies are independent autoantibody systems in RA.

Detection of anti-CCP antibodies
The anti-CCP1 enzyme linked immunosorbent assay (ELISA; Immunoscan RA, Eurodiagnostica, Arnhem, The Netherlands) uses plates coated with highly purified synthetic peptides containing citrulline residues. The test was performed according to the manufacturer’s instructions.

Statistical analysis
Statistical analysis was performed using SPSS 10.0 software (SPSS, Chicago, IL). We used the Wilcoxon signed rank test for paired analysis and the Mann-Whitney U test for comparison of patient groups with and without a marked decrease of the acute phase reactants. Correlations were sought using Spearman’s correlation coefficients ($r_s$). Values of $p<0.05$ were considered significant.

RESULTS
IgM RF, but not anti-CCP antibodies, is modulated by infliximab treatment

IgM RF assay using particles sensitised with rabbit IgG
As shown in table 1 for the total cohort and for different cut off points, the RF titre was significantly reduced during infliximab treatment. Furthermore, the majority of patients with RA showed a decrease in RF titre (n = 32), including a reduction of at least two titre steps in 9/32 patients, whereas in nine patients the RF titre increase, including an increase of at least two titre steps in only one patient, and in 21 patients the RF titres did not change.

IgM RF assay using particles sensitised with human IgG
For this alternative RF assay, a similar, significant reduction in RF titre was observed (as illustrated for the total cohort and for different cut off points in table 1). The majority of patients had a decrease in RF titre (n = 33), including a reduction of at least two titre steps in 19/33 patients, whereas the RF titre increased in 15 patients, including an increase of at least two titre steps in 8/15 patients, and remained unchanged in 14 other patients.

Anti-CCP antibodies
A comparison of the concentrations of anti-CCP antibodies at baseline and after 30 weeks of infliximab treatment showed no significant differences, indicating that anti-CCP antibodies are not modulated by infliximab treatment (as given for the total cohort and for the manufacturer’s cut off point in table 1). Moreover, 38/62 patients with RA showed a decrease in concentrations of anti-CCP antibodies, whereas 24/62 patients had an increase after infliximab treatment. Only 23/38 patients had a marked reduction (at least 20% decrease) in anti-CCP antibody levels, whereas in 13/24 patients the concentrations of anti-CCP antibodies increased by at least 20%.

Furthermore, we observed no correlations between changes in RF and changes in anti-CCP antibodies during infliximab treatment (for RF assay using particles sensitised with rabbit IgG: $r_s = 0.015, p = 0.908$; for RF assay using particles sensitised with human IgG: $r_s = -0.083, p = 0.529$), supporting the hypothesis that RF and anti-CCP antibodies are two independent autoantibody systems in RA.

IgM RF, but not anti-CCP antibodies, is associated with changes in acute phase reactants during infliximab treatment
We further investigated whether IgM RF and anti-CCP antibodies at baseline are predictive of the biological response during infliximab treatment. In patients with increased CRP (>10 mg/l, n = 44) or ESR (>20 mm/1st h, n = 37), we analysed the correlations between titres of autoantibodies at baseline and the percentage change in acute phase reactants.


data table 1

<table>
<thead>
<tr>
<th>Cut off point</th>
<th>Patients (n)</th>
<th>Baseline</th>
<th>Week 30</th>
<th>$p$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM RF (assay using particles sensitised with rabbit IgG)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\geq$ 40</td>
<td>54</td>
<td>320 (40–5120)</td>
<td>160 (0–5120)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>$\geq$ 80</td>
<td>48</td>
<td>320 (80–5120)</td>
<td>160 (0–5120)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>$\geq$ 160</td>
<td>41</td>
<td>320 (160–5120)</td>
<td>160 (0–5120)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Total cohort</td>
<td>62</td>
<td>160 (0–5120)</td>
<td>160 (0–5120)</td>
<td>$&lt;0.001$</td>
</tr>
</tbody>
</table>

| IgM RF (assay using particles sensitised with human IgG) | | | | |
| $\geq$ 40 | 55 | 160 (40–5120) | 160 (0–5120) | 0.038 |
| $\geq$ 80 | 46 | 320 (80–5120) | 160 (0–5120) | 0.017 |
| $\geq$ 160 | 39 | 320 (160–5120) | 160 (0–5120) | 0.023 |
| Total cohort | 62 | 160 (0–5120) | 160 (0–5120) | 0.038 |

Anti-CCP antibodies

<table>
<thead>
<tr>
<th>Cut off point</th>
<th>Patients (n)</th>
<th>Baseline</th>
<th>Week 30</th>
<th>$p$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM RF (assay using particles sensitised with human IgG)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$&gt;50$ U*</td>
<td>41</td>
<td>880 (65–5236)</td>
<td>719 (54–8802)</td>
<td>0.530</td>
</tr>
<tr>
<td>Total cohort</td>
<td>62</td>
<td>535 (5–5236)</td>
<td>447 (7–8802)</td>
<td>0.240</td>
</tr>
</tbody>
</table>

*Manufacturer’s cut off point. Median values (range) are given. Wilcoxon signed rank test.
during infliximab treatment. Table 2 shows that a significant inverse correlation was found between the baseline IgM RF titres measured by an agglutination assay using particles sensitised with human IgG and the percentage change in CRP and ESR, indicating that patients with high baseline IgM RF titres have a less pronounced decrease in acute phase reactants. This was further confirmed by a similar trend using the other RF assay (table 2). In contrast, baseline concentrations of anti-CCP antibodies did not correlate significantly with changes in CRP or ESR during infliximab treatment (table 2).

Furthermore, we analysed the baseline autoantibody titres in the patients with and without a marked decrease of at least 20% in CRP and ESR during infliximab treatment. We observed significantly lower IgM RF titres in the patients with a marked decrease in acute phase reactants than in those with a less pronounced decrease in CRP and ESR (table 3). Again, no statistically significant differences were found in baseline concentrations of anti-CCP antibodies (table 3).

**DISCUSSION**

The importance of the different RA associated antibodies as diagnostic markers for RA has been extensively analysed, and a valid comparison clearly indicates that anti-CCP antibodies show a better diagnostic performance than RF. However, few data are available on the relationship between antibody titres and response to treatment. Some studies describe a decrease in RF titres during successful treatment with methotrexate or parenteral gold, but until now, changes in RF titres during anti-TNFα treatment have only been analysed in two studies (by Maini et al and Charles et al), who reported a decrease in RF titres in infliximab treated patients with RA.

Our study describes the effect of TNFα blockade on both the RF and anti-CCP antibodies in the same cohort of patients with RA. Interestingly, a clearly different effect of infliximab treatment on the IgM RF and anti-CCP antibodies was found: RF titres decreased significantly (confirming the results reported by Maini et al and Charles et al), but concentrations of anti-CCP antibodies did not change. These remarkable findings indicate that anti-CCP antibodies could act as a disease-specific marker for RA and are not modulated by infliximab treatment, whereas IgM RF titres could be related to disease activity as suggested by the decrease in IgM RF titres during infliximab treatment.

Another important topic in view of these new biological treatments such as TNFα blockade is the identification of biomarkers that predict response to treatment. When analysing the RA associated autoantibodies in relation to changes in acute phase reactants, we observed an inverse correlation between the baseline IgM RF titres and the changes in CRP and ESR. In contrast, the baseline concentrations of anti-CCP antibodies did not correlate significantly with the changes in acute phase reactants during infliximab treatment. In other words, patients with RA with high baseline IgM RF titres have a less pronounced decrease of CRP and ESR. These findings were confirmed by the lower baseline RF titres in patients with a marked decrease in acute phase reactants compared with those with a less pronounced decrease in CRP and ESR.

In conclusion, both the differential effect of infliximab treatment on IgM RF and the anti-CCP antibodies, and the different predictive value on the changes in acute phase reactants during infliximab treatment add support to the existing evidence that RF and anti-CCP antibodies are two different, independent autoantibody systems in RA. Our data indicate that the RF and anti-CCP antibodies may provide different and, eventually, complementary biological information on the disease process in RA.

**ACKNOWLEDGEMENTS**

We thank Jeanine Discart for excellent technical assistance. This work was supported by a grant from the “Vlaams instituut voor de bevordering van het wetenschappelijk-technologisch onderzoek in de industrie” (IWT/SB/11127) and a research grant from the “Bijzonder Onderzoeksfonds”, Ghent University.

**REFERENCES**


Clinical Evidence—Call for contributors

Clinical Evidence is a regularly updated evidence-based journal available worldwide both as a paper version and on the internet. Clinical Evidence needs to recruit a number of new contributors. Contributors are healthcare professionals or epidemiologists with experience in evidence-based medicine and the ability to write in a concise and structured way.

Areas for which we are currently seeking authors:

- Child health: nocturnal enuresis
- Eye disorders: bacterial conjunctivitis
- Male health: prostate cancer (metastatic)
- Women’s health: pre-menstrual syndrome; pyelonephritis in non-pregnant women

However, we are always looking for others, so do not let this list discourage you.

Being a contributor involves:

- Selecting from a validated, screened search (performed by in-house Information Specialists) epidemiologically sound studies for inclusion.
- Documenting your decisions about which studies to include on an inclusion and exclusion form, which we keep on file.
- Writing the text to a highly structured template (about 1500–3000 words), using evidence from the final studies chosen, within 8–10 weeks of receiving the literature search.
- Working with Clinical Evidence editors to ensure that the final text meets epidemiological and style standards.
- Updating the text every six months using any new, sound evidence that becomes available.

The Clinical Evidence in-house team will conduct the searches for contributors; your task is simply to filter out high quality studies and incorporate them in the existing text.

To expand the topic to include a new question about once every 12 months.

Updating the text every six months using any new, sound evidence that becomes available.

Documenting your decisions about which studies to include on an inclusion and exclusion form, which we keep on file.

Writing the text to a highly structured template (about 1500–3000 words), using evidence from the final studies chosen, within 8–10 weeks of receiving the literature search.

Working with Clinical Evidence editors to ensure that the final text meets epidemiological and style standards.

Upd