Influence of methotrexate, TNF blockers and prednisolone on antibody responses to pneumococcal polysaccharide vaccine in patients with rheumatoid arthritis

M. C. Kapetanovic, T. Saxne, A. Sjöholm², L. Truedsson², G. Jönsson¹ and P. Geborek

Objective. To compare antibody responses to 23-valent pneumococcal vaccine (Pneumovax®) in controls and patients with established rheumatoid arthritis (RA) treated with TNF blockers, methotrexate (MTX) or a combination of both.

Methods. Patients with RA (n = 149) and healthy controls (n = 47) were vaccinated. Treatment with TNF blockers (etanercept or infliximab) and MTX was given to 50 patients, and 62 patients were treated with TNF blockers alone or with other DMARDs. MTX alone was given to 37 patients. Concentrations of immunoglobulin G (IgG) antibodies against pneumococcal capsular polysaccharides 23F and 6B were measured by enzyme-linked immunoassay before and 4–6 weeks after vaccination. An immune response was defined as a twofold or higher increase in antibody concentration following vaccination.

Results. Prevaccination antibody levels for both 23F and 6B were similar in the patient groups. Antibody concentrations after vaccination increased significantly in all groups. Patients treated with TNF blockers without MTX showed better immune responses than those treated with TNF blockers in combination with MTX (P = 0.037 for 23F and P = 0.004 for 6B) or MTX alone (P < 0.001 for both 23F and 6B). RA patients given MTX alone had the lowest immune responses. Prednisolone treatment did not influence the responses.

Conclusions. Patients treated with TNF blockers and controls showed similar responses to vaccination. In contrast, patients treated with MTX had reduced responses regardless of anti-TNF treatment. The findings do not argue against the use of pneumococcal vaccination in RA patients undergoing treatment with TNF blockers.

KEY WORDS: Pneumococcal vaccination, Antibody response, TNF blockers, Methotrexate, Rheumatoid arthritis, Prednisolone.
clinical evidence of efficacy [1, 2, 20–29]. Also, in immunocompromised patients there is evidence of diminished or absent antibody responses to pneumococcal vaccine in certain groups [2, 30–35].

There are insufficient data from larger controlled trials on RA patients undergoing treatment with TNF-blocking agents and other DMARDs to allow more specific recommendations concerning pneumococcal vaccination. Despite the presumably decreased effectiveness of pneumococcal vaccine in some immunocompromised patients, the potential benefits have so far been judged to justify its use. This study aims to assess the effects of different therapy modalities, including TNF blockers, on the anti-capsular antibody response to vaccination in patients with longstanding RA and to compare the response obtained in healthy controls.

**Material and methods**

All patients treated with TNF blockers at the Department of Rheumatology at Lund University Hospital were offered pneumococcal vaccination without extra cost. To identify control groups, consecutive RA patients taking methotrexate (MTX) without anti-TNF drugs attending the Department of Rheumatology, as well as healthy individuals among staff members (controls) at the Departments of Rheumatology and Infectious Diseases at Lund University Hospital, were offered free vaccination. The study was conducted during the winter seasons 2000/2001 and 2001/2002. Patients were offered pneumococcal vaccination according to the guidelines of the Swedish National Health Board. Ethical approval from local Ethical Review Board at Lund University (LU 513-01) was obtained for vaccination of the medical staff.

Etanercept was given in a dosage of 25 mg subcutaneously twice a week and infliximab as an intravenous infusion in a dosage of 3 mg/kg body weight over 2 h at the start, after 2 and 6 weeks and thereafter as a rule every 8 weeks. The treatments were given on an out-patient basis. For comparison, anti-TNF treated patients were stratified into two groups according to concomitant use of MTX. A group of 62 patients were treated with TNF blockers as monotherapy or combined with DMARDs other than MTX (TNF blockers without MTX’ group). Fourteen of these patients received one other DMARD: four sulphasalazine, five azathioprine, two antimalarial, two oral gold, and one cyclosporin. Fifty patients were treated with anti-TNF treatment combined with MTX (‘TNF blockers + MTX’ group) and 12 of these patients had concomitant therapy with other DMARDs: six sulphasalazine, two cyclosporin, two antimalarial and two both sulphasalazine and antimalarial. In the group of patients treated with MTX without TNF blockers, 10 patients received concomitant DMARDs (five sulphasalazine and five antimalarial). The protocol also included items of diagnosis, age, disease duration, use and dosage of prednisolone and other DMARDs for all patients.

For anti-TNF treated patients, detailed information on disease characteristics and disease activity according to the South Swedish Arthritis Treatment Group protocol (SSATG) [36], including EULAR (European League against Rheumatism) grading of disease activity using the disease activity score (DAS) [37, 38], was available prior to vaccination and before anti-TNF therapy initiation. For the MTX-treated control patients, global visual analogue scale values (VAS-global) were missing and C-reactive protein (CRP) was more frequently available than erythrocyte sedimentation rate (ESR). Therefore, the three variables of the DAS using CRP was calculated for all patients. These DAS values are calculated with the formula:

\[
\text{DAS} = \sqrt{(\text{TJC}) \times 0.56 + \sqrt{\text{SJC}} \times 0.28 + 0.36 \ln(\text{CRP} + 1)} \times 1.1 + 1.15
\]

(for references see www.DAS-score.nl), where TJC is the 28-joint tender joint count, SJC is the 28-joint swollen joint count, and CRP is in mg/l. The DAS values were used to grade disease severity according to EULAR criteria [37, 38], where DAS < 3.2, 3.2–5.1 and > 5.1 correspond to low, intermediate and high disease activity, respectively. Information on healthy volunteers included age and gender.

**Vaccination procedure**

Each participant received a commercially available 23-valent polysaccharide pneumococcal vaccine (Pneumovax; Merck) administrated as a subcutaneous injection in the upper arm with 0.5 ml of a single lot of pneumococcal vaccine containing 25 μg of each of 23 capsular polysaccharide types. Blood samples were obtained before and 4–6 weeks after vaccination and serum was frozen at −20°C. Vaccination in infliximab-treated patients was performed immediately prior to a scheduled infliximab infusion.

**Quantitation of human IgG antibodies specific for S. pneumoniae capsular polysaccharides by ELISA**

The results are presented as the immunization response, i.e. the ratio between post- and prevaccination antibody concentrations. A positive immunization response was defined as a 2-fold or higher increase compared with the prevaccination antibody concentration. Since a protective level of serum antibody has not been strictly defined and may differ among serotypes, these values were not used to identify individuals with probable/possible protective antibody levels.

Levels of serotype-specific pneumococcal IgG to 23F and 6B were measured using the WHO standard enzyme-linked immunosorbent assay (ELISA) for quantitation of human IgG antibodies specific for S. pneumoniae capsular polysaccharides (Pn PS ELISA), as previously described [39]. Briefly, ELISA plates were coated with Pn PS. Dilutions of human sera absorbed with pneumococcal capsular polysaccharide were then added to the ELISA plates. The serotype-specific antibodies (for 23F and 6B) were detected using goat anti-human IgG antibodies conjugated with alkaline phosphatase, followed by addition of the substrate, p-nitrophenyl phosphate. The optical density was measured at 405 nm using an ELISA plate reader. The optical density of the coloured end product is proportional to the amount of anti-capsular PS present in the serum. The assay was calibrated with an international reference serum that was kindly provided by Dr C. Frasch, Bethesda, MD, USA [40]. The lower limit of detection was 0.01 mg/l.

The distributions of the outcome variables were extremely skewed (Shapiro–Wilk W = 0.24–0.74). Therefore, we used non-parametric methods: the Mann–Whitney U-test for comparisons between groups, Wilcoxon’s test for paired variables, and the χ² test for ordinal variables. P values < 0.05 were considered significant.

**Results**

Altogether, 149 patients with established RA and 47 healthy volunteers participated. Patients with ongoing anti-TNF treatment (n = 112) with either etanercept (n = 48) or infliximab (n = 64) and patients with MTX therapy (n = 37) without anti-TNF drugs were vaccinated. Demographic and clinical characteristics of patients and healthy volunteers prior to vaccination are summarized in Table 1. Etanercept was more commonly used without MTX compared with infliximab. Controls were younger and patients in the MTX group were older. Disease duration was longer for TNF blockers without MTX. There were no differences in MTX dosage and duration of MTX treatment before vaccination, and anti-TNF treatment duration was similar between the groups receiving TNF.
Table 1. Demographics and clinical characteristics of patients and healthy controls before vaccination

<table>
<thead>
<tr>
<th>Treatment</th>
<th>I: TNF blockers without MTX</th>
<th>II: TNF blockers + MTX</th>
<th>III: MTX</th>
<th>IV: controls</th>
<th>Significant differences between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>62</td>
<td>50</td>
<td>37</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Infliximab/etanercept (number)</td>
<td>27/35</td>
<td>37/13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>53.7 (15.1–85.3)</td>
<td>52.8 (20.9–80.8)</td>
<td>61.3 (20.8–81.4)</td>
<td>30.3 (19.2–60.3)</td>
<td>P = 0.001 for I↔II, P &lt; 0.001 for I↔IV, H↔IV, and III↔IV</td>
</tr>
<tr>
<td>Disease duration (yr)*</td>
<td>20.8 (1.5–55.9)</td>
<td>10.8 (2.4–39.8)</td>
<td>7.0 (0.9–46.9)</td>
<td>P &lt; 0.001 for I↔III and II↔III</td>
<td></td>
</tr>
<tr>
<td>Female (%)</td>
<td>76</td>
<td>70</td>
<td>68</td>
<td>74</td>
<td>P &lt; 0.001 for I↔II and I↔III</td>
</tr>
<tr>
<td>Concomitant steroids (%)</td>
<td>50.0</td>
<td>52.0</td>
<td>51.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prednisolone dosage (mg/week)*</td>
<td>17.5 (0–105)</td>
<td>6.5 (0–105)</td>
<td>17.5 (0–105)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration (yr)</td>
<td>20.8 (1.5–55.9)</td>
<td>10.8 (2.4–39.8)</td>
<td>7.0 (0.9–46.9)</td>
<td>P &lt; 0.001 for I↔III and II↔III</td>
<td></td>
</tr>
<tr>
<td>Methotrexate treatment duration (yr)*</td>
<td>1.1 (0–1.6)</td>
<td>0.7 (0–1.7)</td>
<td>0.7 (0–1.7)</td>
<td>P &lt; 0.001 for I↔III and II↔III</td>
<td></td>
</tr>
<tr>
<td>Methotrexate dosage (mg/week)*</td>
<td>3.5 (0–10.9)</td>
<td>2.9 (0.1–13.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-TNF treatment duration (yr)*</td>
<td>15 (5–25)</td>
<td>15 (7.5–25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DASb</td>
<td>Low/intermediate/high disease activity at vaccination (%)</td>
<td>49/41/10</td>
<td>50/44/6</td>
<td>53/35/12</td>
<td></td>
</tr>
<tr>
<td>Immunization responsea</td>
<td>76</td>
<td>70</td>
<td>68</td>
<td>74</td>
<td>P &lt; 0.001 for I↔II and I↔III</td>
</tr>
<tr>
<td>Anti-TNF treatment initiation (%)</td>
<td>6/54/40</td>
<td>6/54/40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Groups were as follows: TNF blockers without MTX = anti-TNF treatment with or without DMARD excluding MTX; TNF blockers + MTX = anti-TNF treatment plus MTX with or without other DMARD; MTX = MTX with or without other DMARD excluding TNF blockers; controls = healthy controls. *Median (minimum–maximum). bDAS, disease activity score: low, DAS < 3.2; intermediate, DAS 3.2–5.1; high DAS > 5.1 using the three variables 28-joint swollen joint count (SJC), 28-joint tender joint count (TJC) and CRP in mg/l, where DAS = \((\sqrt{\text{TJC}} \times 0.56 + \sqrt{\text{SJC}} \times 0.28 + 0.36 \ln(\text{CRP + 1})) \times 1.1 + 1.15\).

Table 2. Prevaccination and postvaccination antibody concentrations (mg/l) for 23F and 6B

<table>
<thead>
<tr>
<th>Treatment</th>
<th>I: TNF blockers without MTX</th>
<th>II: TNF blockers + MTX</th>
<th>III: MTX</th>
<th>IV: Controls</th>
<th>Significant differences between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevaccination antibody concentrations</td>
<td>23F 0.7 (0–9.4)</td>
<td>0.8 (0–4.7)</td>
<td>0.8 (0.1–3.8)</td>
<td>1.2 (0–9.7)</td>
<td>I↔IV P = 0.035; II↔IV P = 0.050</td>
</tr>
<tr>
<td></td>
<td>6B 0.9 (0–21)</td>
<td>1.0 (0–7.8)</td>
<td>1.1 (0–8.0)</td>
<td>2.1 (0.1–30)</td>
<td>I↔II P = 0.037; I↔III P = 0.036; II↔IV P = 0.030; III↔IV P = 0.017</td>
</tr>
<tr>
<td>Postvaccination antibody concentrations</td>
<td>23F 2.4 (0.2–18)</td>
<td>1.7 (0.1–19)</td>
<td>1.6 (0.9–1)</td>
<td>2.8 (0.0–103)</td>
<td>III↔IV P = 0.040</td>
</tr>
<tr>
<td></td>
<td>6B 3.8 (0–97)</td>
<td>3.9 (0–63)</td>
<td>2.1 (0–33)</td>
<td>4.6 (0.1–101)</td>
<td>III↔IV P = 0.040</td>
</tr>
<tr>
<td>Immune responsea</td>
<td>23F 2.8 (0.9–68)</td>
<td>2.0 (0.7–36)</td>
<td>1.4 (0.3–15)</td>
<td>2.3 (0.2–91)</td>
<td>I↔II P = 0.037; I↔III P &lt; 0.001; I↔IV P = 0.044; IV↔II P &lt; 0.001; IV↔III P = 0.047</td>
</tr>
<tr>
<td></td>
<td>6B 3.4 (0.8–280)</td>
<td>1.8 (0.9–44)</td>
<td>1.6 (0.8–20)</td>
<td>2.2 (0.4–75)</td>
<td></td>
</tr>
</tbody>
</table>

*aRatio of post- and prevaccination concentrations. All values are given as median (minimum–maximum) values. All values < 0.01 mg/l are taken as 0.

blocks without MTX and TNF blockers + MTX. The number of patients taking prednisolone was similar in all patient groups, and the dosages were not significantly different between the groups. Gender was not significantly different between any of the groups. Disease activity at vaccination was similar in all patient groups, but, compared with the time when anti-TNF was initiated, there had been a significant reduction in the number of patients with high disease activity.

Antibody response

Prior to vaccination almost all participants had detectable levels of antibodies to both 23F and 6B (only two lacked antibodies to 23F, and seven to 6B). Prevaccination antibody concentrations were similar for the different treatment groups but tended to be higher among controls for both 23F and 6B (Table 2). The only significant differences were for 23F between the TNF blockers without MTX group vs controls (P = 0.035) and the TNF blockers + MTX group vs controls (P = 0.050).

Postvaccination antibody concentrations increased significantly in all groups. Median and range values as well as significant differences between the treatment groups are given in Table 2. Immunization responses, i.e. the ratios between post- and prevaccination antibody concentrations, are summarized in Table 2. There were large differences between the groups with regard to immune responses. Responses were highest for TNF blockers without MTX and lowest for MTX. Controls tended to respond better than patients in the MTX group, but this did not reach significance (P = 0.059 for 23F and P = 0.058 for 6B). There were no significant differences between the TNF blockers + MTX and MTX groups (P = 0.065 for 23F and P = 0.246 for 6B).

The proportion of subjects having an immune response, defined as a ≥2-fold increase in the antibody concentration, differed between the treatment groups (Figs 1–3), but essentially followed the same patterns as for immune response ratios. The highest
percentage of immune responses was found in the group receiving TNF blockers without MTX and the lowest in the MTX group.

No significant differences or trends could be traced regarding the influences of gender, age, disease duration, disease activity, anti-TNF treatment duration, MTX treatment duration and dosage or prednisolone use and dosage, when testing subjects with a ≥2-fold increase in antibody concentration vs those with an increase of <2-fold and within the different treatment groups. This was found when testing either 23F or 6B alone, or responders to both 23F and 6B. Among healthy controls, those with a ≥2-fold increase in antibody concentration in response to both 23F and 6B were significantly younger than those lacking a response (median 27 vs 38 yr; \( P = 0.015 \)). This was not statistically significant when testing 23F (\( P = 0.069 \)) or 6B (\( P = 0.138 \)) responders vs non-responders alone. The number of subjects with immune responses to both 23F and 6B stratified according to age and treatment group is shown in Table 3. When testing subjects above 60 yr vs those below 60 yr, no statistical differences were found within any of the patient groups.

**Discussion**

The major findings in this study are that anti-TNF therapy and prednisolone in low doses do not impair the antibody responses following pneumococcal vaccination, while MTX does reduce the response.

It is well known that immune responses to polysaccharides are lower than responses to protein antigens [20, 41]. Furthermore, precise protective levels have not been established [2, 41, 42]. A twofold increase in the antibody level was chosen as an indicator of immune responsiveness [1–3]. Further uncertainties include variation of individual responses to different pneumococcal polysaccharide antigens [42]. This was also illustrated by our findings (Table 2). Our results show a somewhat lower combined responsiveness compared with a recent study in psoriatic arthritis patients [35], but this may merely reflect the choice of antigens analysed. The rationale for choosing 23F and 6B was that they represent two common serotypes known to be associated with invasive infections that are common among patients with underlying diseases in Sweden [19].

The reduced immune response in the MTX group suggests that reduced responsiveness to exogenous or possibly endogenous antigens may be one of the modes of action of MTX that reduces rheumatoid inflammation. Recent reports of anti-rheumatic effects by B-cell depletion in RA patients suggest that decreased antibody production may decrease the rheumatic inflammation [43, 44]. However, the similar MTX dosages and treatment duration times in patients in the TNF blockers + MTX and MTX groups suggest that drug exposure alone is not satisfactory as the sole explanation, since immune responses were different in the two groups. Instead, individually good clinical RA responses to MTX therapy (53% had low disease activity at vaccination in the MTX group; Table 1) seem to indicate reduced immune responsiveness. Almost all
anti-TNF-treated patients had been exposed to MTX prior to anti-TNF therapy, according to guidelines issued by the Swedish Society of Rheumatology. Therefore, patients treated with these remedies represent an RA population with intolerance to or only a partial response to MTX. This is illustrated by the low proportion of patients with low disease activity prior to anti-TNF treatment (6% in the TNF blockers + MTX group; Table 1).

Enhanced immune responses due to anti-TNF treatment are an interesting possibility. There have not been indications of increased total immunoglobulin levels in anti-TNF-treated patients [45], and the findings of increased autoantibody levels in patients undergoing treatment [46–49] could support such a notion. Alternative explanations for the low immune response in the MTX group, such as selection of patients with an inherently good immune response in the other groups, seem less likely. That MTX is the most plausible explanation for the reduced immune response is supported by similar results in reports using other patient groups [32–35].

The analysis of the effect of age on the immune response does not support the notion that high age reduces the likelihood of a positive immune response, at least not among RA patients. An interesting observation in this study is that patients above 60 yr in the group receiving TNF blockers without MTX showed immune responses similar to those of healthy controls below 30 years. However, the analyses of the effect of age at higher ages were not performed due to the restricted number of patients. Because of the statistically different mean ages of the control and treatment groups, an influence of age on the immune response cannot be completely ruled out and may be considered a limitation of this study. By contrast, therapy is important for the immune response in RA patients (Table 3).

The prevaccination presence of antibodies to 6B and 23F polysaccharide antigens presumably reflects antibodies acquired during life as a result of pneumococcal infections. Prevaccination antibody levels were particularly high in some subjects in the healthy control group. Also, prevaccination levels of antibodies to pneumococcal polysaccharides are of limited value for selection of patients suitable for vaccine due to variations between different serotypes. The present findings suggest that pneumococcal vaccination should be performed prior to MTX initiation, whereas anti-TNF treatment and a low prednisolone dose do not preclude vaccination during ongoing therapy. Whether vaccinations actually reduce the true incidence of infectious complications in RA patients should be addressed in future trials.

### Table 3. Immune responses, defined as a ≥2-fold increase in antibody levels for both 23F and 6B in patients grouped according to age and treatment group

<table>
<thead>
<tr>
<th>Response</th>
<th>TNF blockers without MTX</th>
<th>TNF blockers + MTX</th>
<th>MTX</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>&gt;60</td>
<td>12</td>
<td>8</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>50–60</td>
<td>11</td>
<td>12</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>40–50</td>
<td>4</td>
<td>7</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>30–40</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>&lt;30</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Sum</td>
<td>31</td>
<td>31</td>
<td>16</td>
<td>34</td>
</tr>
</tbody>
</table>

### Key message

- Anti-TNF therapy and prednisolone in low doses do not impair the antibody responses following pneumococcal vaccination, while MTX does reduce the response.

### Acknowledgements

The authors thank nurses Elna Haglind and Eva-Karin Kristofferson (Department of Rheumatology, Lund University Hospital) for their help with vaccination and collecting the blood samples and Eva Holmström (Section of Microbiology, Institute of Laboratory Medicine, Lund) for performing the antibody assays. The study was supported by grants from the Swedish Rheumatism Association, the Swedish Research Council (grant no. 15092), the Medical Faculty of the University of Lund, Alfred Osterlund’s Foundation, The Crafoord Foundation, Greta and Johan Kock’s foundation, The King Gustaf V Foundation and Lund University Hospital.

The authors have declared no conflicts of interest.

### References

41. Scheifele DW. Using vaccine responses to plumb the immunologic consequences of tumour necrosis factor factor blockade with etanercept.