**CONCISE REPORT**

**Pneumococcal polysaccharide vaccination in rheumatoid arthritis patients receiving tocilizumab therapy**

Shunsuke Mori,1 Yukitaka Ueki,2 Yukihiro Akeda,3 Naoyuki Hirakata,2 Motohiro Oribe,4 Yoshiki Shiohira,5 Toshikiko Hidaka,6 Kazunori Oishi7

**ABSTRACT**

**Objectives** We assessed the impact of tocilizumab (TCZ), a humanised monoclonal anti-interleukin-6 receptor antibody, on antibody response following administration of the 23-valent pneumococcal polysaccharide vaccine (PPV23).

**Methods** A total of 190 patients with rheumatoid arthritis (RA) received PPV23. Patients were classified into TCZ (n=50), TCZ + methotrexate (MTX) (n=54), MTX (n=62) and RA control (n=24) groups. We measured serotype-specific IgG concentrations of pneumococcal serotypes 6B and 23F using ELISA and functional antibody activity using a multiplexed opsonophagocytic killing assay, reported as the opsonisation indices (OIs), before and 4–6 weeks after vaccination. Positive antibody response was defined as a 2-fold or more increase in the IgG concentration or as a ≥10-fold or more increase in the OI.

**Results** IgG concentrations and OIs were significantly increased in all treatment groups in response to vaccination. The TCZ group antibody response rates were comparable with those of the RA control group for each serotype. MTX had a negative impact on vaccine efficacy. Multivariate logistic analysis confirmed that TCZ is not associated with an inadequate antibody response to either serotype. No severe adverse effect was observed in any treatment group.

**Conclusions** TCZ does not impair PPV23 immunogenicity in RA patients, whereas antibody responses may be reduced when TCZ is used as a combination therapy with MTX.

**INTRODUCTION**

*Streptococcus pneumoniae* (pneumococcus) infection is responsible for substantial mortality and morbidity among adults aged ≥65 years or those with underlying chronic or immunosuppressive conditions. The CDC Advisory Committee on Immunization Practice has recommended the use of the 23-valent pneumococcal polysaccharide vaccine (PPV23) for prevention of invasive pneumococcal disease in at-risk populations.1 Patients with rheumatoid arthritis (RA) are at an increased risk of contracting infectious diseases because of immunological changes that are intrinsic to RA and that result from immunosuppressive agents, and thus it is likely that pneumococcal vaccination can benefit this patient population.

Tocilizumab (TCZ), a humanised monoclonal antibody against the interleukin-6 (IL-6) receptor, is effective and generally well tolerated when administered either as monotherapy or in combination with methotrexate (MTX) in patients with moderate to severe RA. IL-6 was originally identified as a factor essential for B cell differentiation into antibody-producing plasma cells,2 and IL-6-deficient mice had reduced antigen-specific IgG following immunisation with a T-cell-dependent antigen.3 PPV23 induces serotype-specific IgG in a T-cell-independent polysaccharide antigen pathway, which can enhance pneumococcal opsonisation, phagocytosis and killing by phagocytic cells.4 PPV23 immunogenicity is often impaired in certain groups of immunocompromised patients,1 but evidence of PPV23 efficacy and safety is lacking in RA patients receiving TCZ.

The objective of the present study was to evaluate the influence of TCZ therapy on antibody response to PPV23 in RA patients. We determined the serum concentrations of serotype-specific IgG using ELISAs and the functional antibody activity using multiplexed opsonophagocytic killing assays (OPAs) in RA patients being treated with TCZ, MTX or TCZ and MTX, and in control RA patients who received neither drug.

**METHODS**

**Patients**

RA patients who were receiving TCZ therapy (at least the first dose of an intravenous infusion of 8 mg/kg every 4 weeks) and/or MTX (4–18 mg per week) for ≥12 weeks at our rheumatology outpatient clinics were invited to participate in this open-label study. RA patients who had been treated with b uncillamine or salazosulfapyridine were also included as RA controls. All participants fulfilled the 1987 American College of Rheumatology criteria for RA diagnosis. Exclusion criteria were current prednisolone use (≥10 mg/day), current use of immunosuppressive antirheumatic drugs other than MTX (such as tacrolimus, cyclosporine, leflunomide, cyclophosphamide and azathioprine), a recent history (within 6 months) of pneumococcal infection and a history of pneumococcal vaccination. Patients who had changed treatments during the follow-up period or those who had received biological agents other than TCZ were also excluded from this study.

**Vaccine**

We used commercially available PPV23 (Pneumovax NP Merck Sharp & Dohme Corp., Tokyo, Japan) containing 25 μg each of 23 capsular polysaccharide...
RESULTS

Clinical and demographic characteristics
A total of 190 RA patients were divided into four groups according to their ongoing anti-RA therapy. There was one group of 50 patients treated with TCZ as monotherapy (TCZ group), 62 patients treated with MTX alone (MTX group), 54 patients who received a combination therapy consisting of TCZ and MTX (TCZ+MTX group) and 24 patients who did not receive either drug (RA control group). Prior to participating in this study, no patients had received a pneumococcal vaccination. Patients’ clinical and demographic characteristics are shown in table 1.

Table 1. Clinical and demographic characteristics of RA patients prior to pneumococcal vaccination

| Male/female | MTX group (n=62) | TCZ+MTX group (n=54) | TCZ group (n=50) | RA control (n=24) | p Values between treatment groups |
|-------------|------------------|----------------------|------------------|-------------------|----------------------------------|
| Age, mean (95% CI) (years) | 68.3 (66.6 to 70.1) | 65.1 (63.1 to 67.0) | 68.3 (65.8 to 70.8) | 69.2 (65.3 to 73.1) | NS |
| Duration, mean (95% CI) (years) | 10.0 (7.8 to 12.1) | 9.1 (7.3 to 10.8) | 12.5 (9.6 to 15.3) | 11.3 (6.0 to 16.6) | NS |
| MTX dose, median (IQR) (mg/week) | 8 (6 to 8) | 8 (6 to 8) | – | – | NS |
| TCZ duration, median (IQR) (months) | 48 (14.3 to 86.3) | 48.3 (26 to 81) | – | – | NS |
| Use of prednisolone, number of patients (%) | 17 (27.4) | 14 (25.9) | 12 (24) | 1 (4.2) | 0.018 (M vs C) 0.029 (T vs C) 0.049 (T/M vs C) 0.018 (M vs C) 0.094 (T vs C) 0.003 (T/M vs C) 0.001 (T vs C) |
| Prednisolone dose, median (IQR) (mg/day) | 0 (0 to 2) | 0 (0 to 1) | 0 (0 to 1) | 0 (0 to 1) | NS |
| RAN, number of patients (%) | 35 (56.5) | 39 (72.2) | 31 (62) | 8 (33.3) | 0.001 (T vs C) |
| Positive anti-CCP Abs, number of patients (%) | 44 (71.0) | 46 (85.2) | 41 (82) | 11 (45.8) | 0.029 (M vs C) 0.0003 (T vs C) 0.001 (T vs C) |
| Lymphocytes, mean (95% CI) (×10⁹) | 1374 (1230 to 1517) | 1651 (1420 to 1881) | 1717 (1545 to 1890) | 1600 (1358 to 1842) | NS |
| Serum IgG, mean (95% CI) (mg/dl) | 1286 (1194 to 1377) | 1172 (1075 to 1269) | 1196 (1121 to 1271) | 1394 (1258 to 1530) | NS |

Monitoring adverse effects
Adverse events that occurred during a follow-up period of 4–6 weeks after vaccination were recorded. Systemic adverse effects included fever, headache, myalgia, asthenia and fatigue. Local adverse events included pain/tenderness, swelling/induration and erythema at the injection sites.

Antibody response
Fold increases relative to pre-vaccination values (post-vaccination value to pre-vaccination value ratios) were determined. Positive antibody response was defined as a 2-fold or more increase in IgG concentrations or as a 10-fold or more increase in opsonisation indices (OIs).

Opsonophagocytic killing assays
After vaccination, GM-OIs for the 6B and the 23F serotypes were increased significantly in all four groups (p<0.0005; table 2). For serotype 6B, the post-vaccination GM-OI was significantly higher in the TCZ group compared with that in the MTX group (p=0.001). The TCZ group also showed a significantly greater fold increase than did the TCZ+MTX group (p=0.036). For serotype 23F, the TCZ group also showed a significantly higher post-GM-OI than did the MTX group (p=0.027). Increases were twofold or more in all treatment groups, and there were no statistically significant differences.

Statistical analysis
To access the PPV23 immunogenicity in patients in each treatment group, IgG concentrations and OIs before and after vaccination were transformed into logarithmic values. IgG geometric mean concentrations (GMCs) and geometric mean OIs (GM-OIs) were calculated as the exponential of an arithmetic mean of log-transformed values. For details regarding statistical analysis, see online supplementary text.

ELISAs for serotype-specific IgG and multiplexed OPAs
Sera were collected immediately before and 4–6 weeks after vaccination and stored at −30°C until tested. To measure serotype-specific IgG concentrations and functional antibody activity against pneumococcal serotypes 6B and 23F, we performed ELISAs and multiplexed OPAs, respectively. For detailed protocols, see online supplementary text.

Types. From October 2011 to March 2012, each patient received a single dose of vaccine (0.5 ml) subcutaneously in the upper arm. For RA patients receiving TCZ, the vaccination was performed on the same day as the TCZ infusion.
There was a moderate correlation between IgG concentrations and OIs for the 6B and the 23F serotypes (serotype 6B: r=0.623, p<0.0005; serotype 23F: r=0.601, p<0.0005).

**Antibody response rates (percentages of patients with positive antibody response)**

The TCZ group antibody response rates were comparable with those of the RA control group for serotypes 6B and 23F (figure 1).

For the IgG concentration specific to serotype 6B, the antibody response rate was significantly higher in the TCZ group (56%) compared with that in the MTX group (37%) and the TCZ+MTX group (24%, p=0.046 and p=0.0009, respectively; figure 1A). For serotype 23F, there was no significant difference in the antibody response rate among the four treatment groups (Control: 67%; MTX: 57%; TCZ+MTX: 56%; TCZ: 72%). The percentage of patients with positive antibody response for both strains were significantly higher in the TCZ group (46%) compared with those in the MTX group (37%, p=0.027) and the TCZ+MTX group (35%, p=0.020). For both strains, a higher proportion of patients in the TCZ group responded to pneumococcal vaccination compared with the patients being treated with MTX alone (34% vs 16%, p=0.028).

**Predictive factors for antibody response to PPV23**

In a multivariate logistic regression analysis, TCZ use was not identified as the predictive factor for antibody response to pneumococcal vaccination for either IgG concentrations or OIs. The negative association of current MTX use with antibody response was confirmed for IgG concentrations specific to serotypes 6B and 23F (for serotype 6B: OR 0.45, 95% CI 0.25 to 0.82, p=0.009; for serotype 23F: OR 0.56, 95% CI 0.31 to 1.04, p=0.007) and OIs for serotype 23F (OR 0.54, 95% CI 0.29 to 0.99, p=0.046).

**Vaccination safety**

Two patients in the TCZ+MTX group had a fever. Local adverse events were observed in 12 patients (2 in the MTX group, 7 in the TCZ+MTX group and 3 in the TCZ group). All adverse effects were mild.

**DISCUSSION**

Following immunisation with PPV23, IgG concentrations and OIs for the 6B and the 23F serotypes were significantly increased in all treatment groups. Antibody response rates in the TCZ group were comparable with those of the RA control group for each serotype. Ongoing use of MTX is likely to have affected the antibody response to PPV23.

Results of the present study indicate that TCZ does not diminish T-cell-independent antibody production after PPV23 immunisation. In addition, we recently reported that RA patients receiving TCZ can produce an adequate antibody response to influenza vaccine, which are T-cell-dependent protein antigens.6 These findings suggest that both T-cell-dependent and T-cell-independent antibody response pathways are conserved in RA patients who are treated with TCZ. There is an increasing awareness of lethal synergism between influenza virus and pneumococcus; influenza virus contributes to secondary pneumococcal pneumonia and can subsequently increase mortality.7-8 In addition, a large-scale trial suggested that a significant
In the present study, no patients were receiving high doses of prednisolone or antiarheumatic agents with immunosuppressive effects other than MTX. In addition, there were no differences in the prednisolone dose among the four treatment groups, and the median dose of prednisolone was zero among all groups. The number of prednisolone users was significantly lower in the RA control group; however, there were no significant differences or trends in antibody response to each serotype compared with the other three groups. We can, therefore, say that the influence of such agents on PPV23-induced antibody response was minimal in the present study.

One limitation of this study is the relatively small number of patients in each group and the RA control group in particular. Since most RA patients had already received one or more immunosuppressive antiarheumatic drugs, as recommended by the current therapeutic guidelines, it was difficult to recruit a sufficient number of patients who had never received such drugs. Another limitation is that we determined antibody response to only two pneumococcal serotypes. We chose serotypes 6B and 23F because these are the main causative serotypes of pneumococcal pneumonia in Japan and these are representative penicillin-resistant pneumococci. However, the immune response to PPV23 may not be consistent among the 23 serotypes. Lastly, unlike influenza vaccines, antibody levels that are protective against invasive pneumococcal disease in adults have not been clearly defined. We used a 2-fold increase in the IgG concentration or a 10-fold increase in the OI as a measure of positive antibody response to PPV23 in this study, which was also used in previous studies; however, how this threshold may best correlate with protection against invasive pneumococcal disease remains to be determined.

In conclusion, ongoing TCZ therapy does not preclude pneumococcal polysaccharide vaccination in RA patients; however, antibody responses may be reduced when TCZ is administered in combination with MTX.

Acknowledgements The authors are grateful to Michiyo Hayakawa and Yumi Hattori for technical assistance in measuring serotype-specific IgG concentrations and OIs.

Contributors All authors contributed to study conception and design, acquisition of data, analysis and interpretation of data, and drafting of the manuscript with regard to important intellectual content.

Funding The study was supported by research grants from the Ministry of Health, Labour and Welfare of Japan and research funds from the National Hospital Organization (NHO), Japan.

Competing interests TH has received lecture fees from Mitsubishi-Tanabe Pharmaceutical Co., Eisai Co. Ltd. and Abbott Japan Co. Ltd. The other authors have no financial relationships that could lead to a conflict of interest.

Patient consent Obtained.

Ethics approval The ethics committees of participating hospitals approved the protocol for this study.

Provenance and peer review Not commissioned; externally peer reviewed.

Open Access This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 3.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/3.0/

REFERENCES

1 Advisory Committee on Immunization Practices. Prevention of pneumococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recom Rep 1997;46:1–24.

2 Muraguchi A, Hirano T, Tang B, et al. The essential role of B cell stimulatory factor 2 (BSF-2/IL-6) for the terminal differentiation of B cells. J Exp Med 1988;167:332–44.

3 Kopf M, Baumann H, Freier G, et al. Impaired immune and acute-phase responses in interleukin-6-deficient mice. Nature 1994;368:339–42.
4 Mond JJ, Vos Q, Lees A,, et al. T cell independent antigens. Curr Opin Immunol 1995;7:349–54.
5 Dransfield MT, Nahm MH, Han MK,, et al. Superior immune response to protein-conjugate versus free pneumococcal polysaccharide vaccine in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2009;180:499–505.
6 Mori S, Ueki Y, Hirakata N,, et al. Impact of tocilizumab therapy on antibody response to influenza vaccine in patients with rheumatoid arthritis. Ann Rheum Dis 2012;71:2006–10.
7 McCullers JA, Rehg JE. Lethal synergism between influenza virus and Streptococcus pneumoniae: characterization of a mouse model and the role of platelet-activating factor receptor. J Infect Dis 2002;186:341–50.
8 Peltola VT, Murti KG, McCullers JA. Influenza virus neuraminidase contributes to secondary bacterial pneumonia. J Infect Dis 2005;192:249–57.
9 Madhi SA, Klugman KP. A role for Streptococcus pneumoniae in virus-associated pneumonia. Nat Med 2004;10:811–13.
10 Mease PJ, Ritchlin CT, Martin RW,, et al. Pneumococcal vaccine response in psoriatic arthritis patients during treatment with etanercept. J Rheumatol 2004;31:1356–61.
11 Kapetanovic MC, Saxne T, Sjoholm A,, et al. Influence of methotrexate, TNF blockers and prednisolone on antibody responses to pneumococcal polysaccharide vaccine in patients with rheumatoid arthritis. Rheumatology (Oxford) 2006;45:106–11.
12 Visvanathan S, Keenan GF, Baker DG,, et al. Response to pneumococcal vaccine in patients with early rheumatoid arthritis receiving infliximab plus methotrexate or methotrexate alone. J Rheumatol 2007;34:952–7.
13 Gelinck LB, van der Bijl AE, Visser LG,, et al. Synergistic immunosuppressive effect of anti-TNF combined with methotrexate on antibody responses to the 23 valent pneumococcal polysaccharide vaccine. Vaccine 2008;26:3528–33.
14 Recommendations to assure the quality, safety and efficacy of pneumococcal conjugate vaccines. Replacement of: TRS 927, Annex 2. In: WHO Expert Committee on Biological Standardization. Geneva: World Health Organization, 2009:1–57.
15 Kapetanovic MC, Roseman C, Jonsson G, et al. Antibody response is reduced following vaccination with 7-valent conjugate pneumococcal vaccine in adult methotrexate-treated patients with established arthritis, but not those treated with tumor necrosis factor inhibitors. Arthritis Rheum 2011;63:3723–32.
16 Singh JA, Punst DE, Bharat A, et al. 2012 update of the 2008 American College of Rheumatology recommendations for the use of disease-modifying antirheumatic drugs and biologic agents in the treatment of rheumatoid arthritis. Arthritis Care Res (Hoboken) 2012;64:625–39.
17 Elkayam O, Paran D, Caspi D,, et al. Immunogenicity and safety of pneumococcal vaccination in patients with rheumatoid arthritis or systemic lupus erythematosus. J Influenzae 2002;24:147–53.
18 Coulson E, Saravanam V, Hamilton J,, et al. Pneumococcal antibody levels after pneumovax in patients with rheumatoid arthritis or systemic lupus erythematosus. Ann Rheum Dis 2011;70:1289–91.
19 Heijstek MW, Ott de Bruin LM, Bijl M, et al. EULAR recommendations for vaccination in paediatric patients with rheumatic diseases. Ann Rheum Dis 2011;70:1704–12.
20 Oishi K, Yoshimine H, Watanabe H, et al. Drug-resistant genes and serotypes of pneumococcal strains of community-acquired pneumonia among adults in Japan. Respirology 2006;11:429–36.