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

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ABSTRACT

Objective. To compare antibody responses to 23-valent pneumococcal vaccine (Pneumovax®) in controls and patients with established 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, while 62 patients were treated with TNF-blockers alone or with other DMARDs. MTX alone was given to 37 patients. Concentrations of IgG antibodies against pneumococcal capsular polysaccharides 23F and 6B were measured with ELISA prior to and 4-6 weeks after vaccination. An immune response was defined as a twofold or higher increase of antibody concentrations following vaccination.

Results. Pre vaccination 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 methotrexate showed better immune responses than those treated with TNF-blockers in combination with MTX ($p < 0.05$ for 23F and $p < 0.01$ 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

Introduction

Infection caused by *Streptococcus (S.) pneumoniae* is a major cause of mortality and morbidity throughout the world. To prevent severe pneumococcal infections and their complications the Swedish National Board of Health and Welfare and the CDC Advisory Committee on Immunisation Practice recommend immunisation with 23-valent pneumococcal polysaccharide vaccine in persons >65 years of age and patients suffering from chronic illness at high risk for invasive pneumococcal disease (1,2,3).

In patients with rheumatoid arthritis (RA) infections cause significant morbidity and mortality. Possible explanations include immune dysfunction associated with the disease itself, co-morbid illnesses and/or concomitant medication such as immunosuppressive drugs including long-term systemic glucocorticoids (4-10). The incidence of objectively confirmed infections in patients with RA was found to be increased compared to age- and sex matched subjects without RA. Also infections requiring hospitalisation including bacteremia/septicemia and pneumonia were reported to be significantly more frequent in RA patients (4). The use of disease modifying anti-rheumatic drugs (DMARDs) including concomitant treatment with glucocorticoids has been found to be associated with increased risk for infections (7).

Recently the introduction of anti-TNF treatments have contributed to a somewhat changed pattern of infections in RA (11-13). More common infections, such as upper respiratory tract infections, have also been described among common adverse events and reasons for withdrawal of anti-TNF therapy in clinical trials as well as in observational studies (8,14). Doubled rates of serious infectious have also been reported (15). Case reports of serious infections due to *S. pneumoniae* during anti-TNF therapy include pneumonia, severe pneumonia and necrotising fasciitis (infliximab) (16,17), and fatal septicaemia (etanercept) (18).

Commercially available 23-valent pneumococcal capsular polysaccharide vaccine contains 23 purified capsular polysaccharide antigens of *S. pneumoniae* and the vaccine covers at least 85-90 % of the serotypes causing invasive pneumococcal infections (2). The eight most common serotypes of invasive *S. pneumoniae* isolates reported to the Swedish Institute for Infectious Disease Control during 1988-1998 were serotypes: 14, 7F, 9V, 4,3,1, 23F, and 6B (19).

Applying the CDC Advisory Committee on Immunisation Practice recommendations on patients with rheumatic disease, pneumococcal vaccination should be considered and encouraged in a majority of the patients in spite of convincing 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 is 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 at assessing 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 in Lund were offered pneumococcal vaccination without extra costs. To identify control groups consecutive RA patients taking methotrexate without anti-TNF drugs (MTX) attending the Department of Rheumatology, as well as healthy individuals in the staff members (Controls) at the Departments of Rheumatology and Infectious Diseases 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 dosage of 3 mg/kg body weight over 2 hours at start, after two and six 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 methotrexate. A group of 62 patients treated with TNF-blockers as monotherapy or combined with DMARDs other than methotrexate (TNF-blockers without MTX). 14 of these patients received one other DMARD : 4 sulphasalazine, 5 azathioprine, 2

antimalaria, 2 oral gold , and 1 cyclosporine. Fifty patients were treated with anti-TNF treatment combined with methotrexate (TNF-blockers + MTX group) and 12 of these patients had concomitant therapy with other DMARDs: 6 sulphasalazine, 2 cyclosporin, 2 antimalaria and 2 patients both sulphasalazine and antimalaria. In the group patients treated with methotrexate without TNF-blockers (MTX) 10 patients received concomitant DMARDs (5 sulphasalazine and 5 antimalaria). 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 of disease characteristics and disease activity according to the South Swedish Arthritis Treatment Group protocol (SSATG) (36), including EULAR 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 methotrexate treated control patients global visual analogue scale values (VAS-global) were missing and C-reactive protein (CRP) were more frequently available than erythrocyte sedimentation rate (ESR). Therefore the 3 variables of the DAS using CRP was calculated for all patients. These DAS values are calculated with the formula $DAS=[\sqrt{TJC} \cdot 0.56 + \sqrt{SJC} \cdot 0.28 + 0.36 \cdot \ln(CRP+1)] \cdot 1.1 + 1.15$ (For references see www.DAS-score.nl), where TJC=28 joint tender joint count, SJC=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 $DAS < 3.2$, DAS between 3.2 and 5.1, and $DAS > 5.1$ corresponds 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) administered as a subcutaneous injection in the upper arm with 0.5 ml of a single lot of pneumococcal vaccine containing 25 microgram of each of 23 capsular polysaccharides 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 immunization response i.e. the ratio between post- and pre-vaccination concentrations. A positive immunization response was defined as a twofold or higher increase of 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 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. Calibration of the assay was made 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, the Wilcoxon's test for paired variables, and the X^2 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 methotrexate 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 methotrexate compared to infliximab. Controls were younger and MTX were older. Disease duration was longer for TNF-blockers without MTX. There were no differences in methotrexate dosage, and duration of methotrexate treatment before vaccination, and anti-

TNF treatment duration was similar between TNF-blockers 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 lacking antibodies to 23F, and seven to 6B). Prevacination 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 versus Controls ($p=0.035$) and TNF-blockers+ MTX 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 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 MTX but this did not reach significance; $p=0.059$ for 23F and 0.058 for 6B. There were no significant differences between TNF-blockers+ MTX versus MTX with $p=0.065$ for 23F and $p=0.246$ for 6B.

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

No significant differences or trends could be traced regarding influence of gender, age, disease duration, disease activity, anti-TNF treatment duration, methotrexate treatment

duration and dosage or prednisolone use and dosage, when testing subjects with a ≥ 2 -fold increase in immune responses ratio versus those with a < 2 fold increase 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 ≥ 2 -fold increase in immunisation response to both 23F and 6B were significantly younger, median 27 years versus 38 years among those lacking a response ($p = 0.015$). 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 are shown in table 3. When testing subjects above 60 years versus below 60 years, 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 methotrexate does reduce the response.

It is well known that immune responses to polysaccharides are lower than to protein antigens (20, 41). Furthermore, precise protective levels have not been established (2,41,42). A twofold increase of 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 methotrexate which 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 methotrexate dosages and treatment duration times in TNF-blockers+MTX and MTX treated patients 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 methotrexate therapy (53 % low disease activity at vaccination in MTX, table 1) seem to indicate reduced immune responsiveness. Almost all anti-TNF treated patients had been exposed to methotrexate 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 partial response to methotrexate. This is illustrated by the low proportion of patients with low disease activity prior to anti-TNF treatment (6 % in TNF-blockers+MTX, 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 inherent good immune response in the other groups seem less likely. That methotrexate 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 age influence on 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 years in TNF-blockers without MTX group showed similar immune responses as healthy controls below 30 years. However, the analyses of age influence in higher ages were not performed due the restricted number of patients. Because of the statistically different mean age of the control and treatment groups the age influences on the immune response can not be completely ruled out and may be considered as a limitation of this study. By contrast, therapy is important for the immune response in RA patients (table 3).

The pre-vaccination presence of antibodies to 6B and 23F polysaccharide antigens presumably reflect 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 vaccination due to variations between different serotypes. The current findings suggest that pneumococcal vaccination should be performed prior to methotrexate 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.

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Figure legend

Figure 1. Percentage (%) of patients with immune responses defined as a ≥ 2 -fold increase in antibody levels to 23F. The groups were TNF-blockers without MTX = anti-TNF treatment \pm DMARD excluding methotrexate, TNF-blockers +MTX = anti-TNF treatment + methotrexate \pm other DMARD, MTX = methotrexate \pm other DMARD excluding TNF blockers, and Control=healthy controls. Also levels of significance between groups are shown.

Figure 2. Percentage (%) of patients with immune responses defined as a ≥ 2 -fold increase in antibody levels to 6B. For groups see figure 1. Significant differences are also shown.

Figure 3. Percentage (%) of patients with immune responses defined as ≥ 2 -fold increase in antibody levels to both 23F and 6B. For groups see figure 1. Significant differences are also shown.

Table 1: Demographics and clinical characteristics of patients and healthy controls prior to vaccination. The groups were TNF-blockers without MTX = anti-TNF treatment ± DMARD excluding methotrexate. TNF-blockers +MTX = anti-TNF treatment + methotrexate ± other DMARD, MTX = methotrexate ± other DMARD excluding TNF blockers, and controls=healthy controls.

Treatment	I TNF-blockers without MTX	II TNF-blockers +MTX	III MTX	IV Controls	Significant differences between I- IV
Number	62	50	37	47	
Infliximab/etanercept (nr)	27/35	37/13			p=0.001 for I↔II
Age (years)#	53.7 (15.1- 85.3)	52.8 (20.9-80.8)	61.3 (20.8-81.4)	30.3 (19.2-60.3)	p<0.001 for I↔IV, II↔IV, and III↔IV p<0.001 for I↔III and II↔III
Disease duration (years)#	20.8 (1.5-55.9)	10.8 (2.4-39.8)	7.0 (0.9-46.9)		p<0.001 for I↔II and I↔III
Female (%)	76	70	68	74	
Concomitant steroids (%)	50.0	52.0	51.4		
Prednisolone dosage(mg/week)#	17.5 (0-105)	6.5 (0-105)	17.5 (0-105)		
Anti-TNF treatment duration (years) median (min-max) #	1.1 (0-1.6)	0.7 (0-1.7)			
Methotrexate treatment duration (years)#		3.5 (0-10.9)	2.9 (0.1-13.0)		
Methotrexate dosage mg/week #		15 (5-25)	15 (7.5-25)		
Low/Intermediate/High* disease activity at vaccination (%)	49/41/10	50/44/6	53/35/12		
Low/Intermediate/High* disease activity at anti- TNF initiation (%)	6/54/40	6/54/40			

DAS= Disease Activity Score. Low=DAS<3.2, Intermediate =DAS between 3.2 and 5.1, High=DAS>5.1 using the 3 variables 28 joint swollen joint count (SJC), 28 joint tender joint count (TJC) and CRP in mg/L where $DAS = [\sqrt{TJC} * 0.56 + \sqrt{SJC} * 0.28 + 0.36 * \ln(CRP + 1)] * 1.1 + 1.15$
= median (min-max)

Table 2. Prevaccination and postvaccination antibody concentrations (mg/L) for 23F and 6B. The immunisation response i.e. the ratio of post- and prevaccination concentrations is also given. All values are given as median (min-max) values. All values < 0.01 mg/L are indicated with 0.

	I TNF-blockers without MTX	II TNF-blockers +MTX	III MTX	IV Controls	Significant differences between I-IV
Prevaccination antibody concentrations					
23F	0.7 (0-9.4)	0.8 (0-4.7)	0.8 (0.1-3.8)	1.2 (0-9.7)	I↔IV p=0.035; II↔IV p=0.050;
6B	0.9 (0-21)	1.0 (0-7.8)	1.1 (0-8.0)	2.1 (0.1-30)	
Postvaccination antibody concentrations					
23F	2.4 (0.2-18)	1.7 (0.1-19)	1.6 (0-9.1)	2.8 (0.0-103)	I↔III p<=0.036, II↔IV p=0.030, III↔IV p=0.017
6B	3.8 (0 -97)	3.9 (0.1-63)	2.1 (0-33)	4.6 (0.1-101)	III↔IV p=0.040
Immunisation response (post-/prevaccination concentrations)					
23F	2.8 (0.9-68)	2.0 (0.7-36)	1.4 (0.3-15)	2.3 (0.2-91)	I↔II p=0.037, I↔III p<0.001
6B	3.4 (0.8-280)	1.8 (0.9-44)	1.6 (0.8-20)	2.2 (0.4-75)	I↔II p=0.004, I↔III p<0.001; I↔IV p=0.047

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.

Response	TNF-blockers without MTX		TNF-blockers +MTX		MTX		Controls	
	yes	no	yes	no	yes	no	yes	no
>60	12	8	5	11	4	20	0	3
50-60	11	12	8	10	0	10	2	5
40-50	4	7	1	5	1	1	2	4
30-40	3	3	1	6	0	0	1	8
<30	1	1	1	2	0	1	13	9
Sum	31	31	16	34	5	32	18	29

KEY MESSAGES

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





