Association of RF and Anti-CCP Positivity, but not carriage of shared epitope or PTPN22 susceptibility variants, with Response to Anti-TNF Treatment in RA

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Abstract

Objective: To determine whether rheumatoid factor (RF), anti-CCP antibodies, or carriage of shared epitope (SE) and *PTPN22* genetic susceptibility variants predict response to anti-TNF therapy in a large cohort of patients with rheumatoid arthritis (RA).

Methods: UK-wide multi-centre collaborations were established to recruit a large cohort of patients treated with anti-TNF drugs for RA. Serum RF, anti-CCP antibody and SE status were determined using commercially available kits. In addition, *PTPN22* R620W genotyping was performed using a Sequenom MassArray® platform. Linear regression analyses were performed to investigate the role of these 4 factors in predicting response to treatment by 6 months, defined as the absolute change in DAS28. Any effects observed across the entire cohort were explored further to determine whether these factors were better predictors of response to one or other of the anti-TNF agents.

Results: Of the 642 patients analysed, 46% received infliximab, 43% etanercept and 11% adalimumab. Eighty nine per cent were RF positive and 82% were anti-CCP positive. The mean baseline DAS28 was 6.7 and by 6-months had improved by a mean of 2.5 units. Patients who tested negative for RF had a 0.49 (95% CI 0.1, 0.9) greater mean improvement in DAS28 compared to RF positive patients. A better response was also seen among patients who tested negative for anti-CCP. Upon stratification, the association of both RF and anti-CCP antibody status was restricted to the infliximab treatment group. No association was demonstrated between drug response and SE or *PTPN22* 620W carriage, under any model.

Conclusion: The presence of RF or anti-CCP antibodies was associated with a reduced response to anti-TNF drugs as a whole and infliximab, in particular. However, these antibodies only account for a small proportion of the variance in treatment response. It is likely that genetic factors will contribute to the response to treatment but these do not include the 2 genes known to confer susceptibility to RA.

Introduction

The identification of the key role played by tumour necrosis factor (TNF) in the pathogenesis of rheumatoid arthritis (RA) resulted in the development of new therapies that target and block the action of this cytokine. Consequently, 3 such anti-TNF biologic agents have been approved to date, namely etanercept (Enbrel; Amgen/Wyeth), infliximab (Remicade; Centocor/Johnson & Johnson/Schering-Plough) and more recently adalimumab (Humira; Abbott) (1). Collectively these drugs have become one of the most effective methods of treating RA, with nearly half of all treated patients achieving an American College of Rheumatology 20% (ACR20) improvement level or higher. Furthermore, in addition to reducing disease activity, these agents have been reported to inhibit radiological progression (2).

However, there is still a substantial proportion of patients who show partial or no response to anti-TNF therapy. As treatment with such biologic agents is limited by expensive annual costs in many countries, there is a clinical need to identify methods of prospectively determining those patients most, or indeed least, likely to benefit.

A number of demographic and disease specific factors have been examined, but few predictors of response have been consistently identified (3-5). In particular, analyses in a large scale longitudinal observational study cohort identified higher baseline health assessment questionnaire (HAQ) scores and current smoking status as predictors of lower response rates, although this latter factor was only significant in the group of patients treated with infliximab (5). Age, disease duration and number of previous disease modifying anti-rheumatic drugs (DMARDs) did not predict response to either infliximab or etanercept. Several smaller studies (sample sizes < 130) have also investigated the utility of auto-antibodies, including rheumatoid

factor (RF) and anti-cyclic citrullinated peptide (anti-CCP), for predicting response to treatment with a biologic agent but results have been inconsistent (3;4;6;7).

Genetic factors are also likely to play a key role in determining treatment response. Although previous studies have had limited success in identifying genetic predictors, most of these studies were hindered by small sample sizes resulting in limited power to detect modest effects. For instance, association of the *TNF* gene promoter polymorphism at position -308 with response to infliximab was reported in 4 small European populations (n<90) (8-11), but was not replicated in a larger cohort of 198 French patients with RA (12). Similarly, a significant association was demonstrated between carriage of the shared epitope (SE) and response to etanercept in a large cohort of 200 US patients (13) but was not replicated in a smaller cohort of 123 Swedish patients (14). Furthermore, no association of SE status with response to infliximab treatment was observed in two European populations (n= 78 Spanish and 198 French patients, respectively) (12;15). Whether this latter difference could be explained by varying pharmacokinetics/dynamics between the two drugs remains to be determined. Additional studies have investigated polymorphisms in the genes encoding IL1β and the IL1-receptor antagonist (12;14), IL10 (14;16), TGFβ1 (14) and FcγRIIIA (17;18), but few have identified consistent associations with response to anti-TNF treatment response.

Thus, in order to aid the identification of genetic predictors of response, we established a UK-wide multi-centre collaboration to recruit a large cohort of patients treated with anti-TNF biologic therapies for RA. Here we present analyses investigating the role of RF status, anti-CCP status, and carriage of the SE in determining response to treatment. Carriage of the *PTPN22**620W variant, a now well-established RA susceptibility factor, was also investigated.

Methods

Study design

Anti-TNF (etanercept, infliximab, adalimumab) treated patients were recruited from rheumatology centres throughout the UK. In a subset for which DNA had been extracted, a within-cohort association study was performed to investigate the role of RF, anti-CCP antibodies, SE carriage and *PTPN22**620Wcarriage in predicting response to treatment at 6 months. Interaction analyses were also performed to determine whether any effects observed were better predictors for one or other of the anti-TNF drugs.

Patient selection – UK-wide multi-centre collaborations were established to recruit a large cohort of patients treated with anti-TNF drugs for RA. Eligible patients from each centre were subsequently identified from the British Society of Rheumatology's (BSR) Biologics Register (BR) (19). This register compiles extensive clinical information on patients starting treatment with a biologic agent and follows them prospectively, on a 6-monthly basis for 5 years, in order to monitor and determine the incidence of potential short and long term hazards. The following criteria were used for the selection of patients: 1) currently actively participating in the BSRBR long-term safety study, 2) physician-confirmed diagnosis of RA, 3) currently or have been treated with one of the 3 anti-TNF biologic agents, 4) European Caucasian descent and 5) reached 6 months of follow-up. Patients who stopped treatment temporarily during the first 6 months of therapy were excluded from selection. Similarly, patients who discontinued therapy prior to the 6 month follow-up for any reason other than inefficacy were excluded from selection.

Patient recruitment and sample collection – Patients from each collaborating centre were identified from the BSRBR and, through an initial mailing, invited to take part in the study by

the relevant consultants. Additional blood samples were obtained from consenting patients when they attended local rheumatology clinics for blood tests required as part of routine care. The additional blood samples and signed consent forms were subsequently posted back to the Arthritis Research Campaign (**arc**) unit at the University of Manchester for processing and storage. For the majority of patients two samples of blood were taken, one plain and one EDTA tube, from which serum and DNA could be extracted, respectively. DNA was isolated using a standard phenol/chloroform extraction method. Both serum and DNA samples were stored at -80°C.

UK Central Office of Research Ethics Committees (COREC) approval (04/Q1403/37) was obtained for the collection of DNA and serum samples from patients receiving anti-TNF therapy for the treatment of RA.

Clinical information - Clinical data held on the BRSBR database was extracted, with the consultants' permission, and compiled for each consenting patient and included: gender, date of birth, year of disease onset, details of the American College of Rheumatology (ACR) classification criteria for RA (20), smoking status, Health Assessment Questionnaire (HAQ) score (21), details of biologic and non-biologic anti-rheumatic drug therapy (including drug type, changes to therapy, reasons for discontinuation and previous biologic therapy). Disease activity, measured using the 28-joint count disease activity score (DAS28) was extracted at baseline and at 6-months follow-up (22).

Immunogenetics – Serum RF and anti-CCP antibody titre were measured using commercially available kits (RF-PAIA Immunoturbidimetric Assay for rheumatoid factor, Diastat™ Anti-CCP Kit (Axis-Shield Diagnostics Limited, UK)). Patients with titres greater than or equal to

40units/ul and 5units/ul were defined as positive for RF and anti-CCP antibodies, respectively. HLA-DRB1 typing was performed using commercially available kits (Dynal RELI™ SSO HLA-DRB1 Typing Kit (Dynal Biotech Limited, UK)). The SE was defined as the presence of any of the following alleles: HLA-DRB1*0101, *0102, *0104, *0401, *0404, *0405, *0408 or *1001. In addition, *PTPN22* R620W (1858C/T) genotyping was performed using MassARRAY® iPLEX™ assays, followed by matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry (MS) (Sequenom, Cambridge, UK). Assays were designed and performed as recommended by the manufacture (PCR primer 1: ACGTTGGATGACTGAACTGTACTCACCAGC, PCR primer 2: ACGTTGGATGAGATGATGAAATCCCCCCTC, iPLEX extension primer CCCCTCCACTTCCTGTA, extension direction: R).

Analysis

The primary outcome measure was change in DAS28 between baseline and 6-months. Linear regression analyses were performed to investigate association between change in DAS28 and the 4 factors under investigation; namely, RF and anti-CCP status, SE and *PTPN22**620W carriage. For the purposes of this analysis, the recorded 6 month DAS28 score was used whether patients had discontinued therapy or not. Analyses were adjusted for baseline DAS28, baseline HAQ score, administration of concurrent DMARDs and gender, as these factors have previously been shown to be significant independent predictors of response (5). Analyses were repeated excluding any patients with previous exposure to a biologic drug, whether or not it was the same agent. In addition, interaction analyses were performed to determine whether any observed effects were similar across the two major drug types, namely etanercept and infliximab. Patients treated with adalimumab were excluded from this latter analysis due to the small numbers in this subgroup.

Results

Patient recruitment

Collaborations were established with 20 rheumatology centres across the UK from which 1,485 patients receiving anti-TNF therapy for RA were eligible for recruitment based on the criteria outlined. Of these patients, 1,292 responded to the invitation letter (87%) with 1,195 patients willing to take part (80%). DNA samples were extracted and available for the first set of 642 patients to be recruited, which were utilized in the current analysis.

Baseline characteristics and immunogenetics

Baseline characteristics for the group of 642 patients are presented in Table 1. Across this group; 296 patients had received infliximab (46%), 278 etanercept (43%) and 68 adalimumab (11%). The proportion of females, mean age, disease duration, baseline DAS28 and HAQ scores were comparable across all drug types. In addition, these measures were comparable to previous data reported on the BSRBR as a whole (23). A smaller proportion of patients receiving adalimumab were current or previous smokers compared to those receiving either etanercept or infliximab. Across the combined cohort, 41% and 73% of patients were receiving concurrent steroids and single/combined DMARD therapy, respectively. A substantially greater proportion of patients receiving infliximab were receiving concurrent DMARD therapy, compared to those being treated with either etanercept or adalimumab. Finally, a small proportion of patients were recorded as having previous exposure to a biologic agent (5%).

Genotyping of the *PTPN22* R620W (C1858T) polymorphism was successfully performed in 96% of the samples, with 30% being carriers of the minor T risk allele (Table 2). Of those that were successfully typed at the *HLA-DRB1* locus, 81% were defined as SE positive (Table 2). In

addition, for subjects for whom serum samples were available, 89% and 82% were positive for RF and anti-CCP antibodies, respectively (Table 2).

Predictors of response

By the first 6 months follow-up, 90% of this group of patients were still receiving anti-TNF therapy. The remaining 10% had discontinued treatment due to inefficacy. In total, 21% of patients were non-responders, 52% moderate responders and 27% good responders, based on the European League Against Rheumatism (EULAR) improvement criteria (24). The mean change in DAS28 was an improvement of 2.5 points. Both baseline and absolute change in DAS28 values were normally distributed across the patient population.

Regression analyses were first performed to investigate association between drug response and baseline factors including concurrent DMARD therapy, HAQ score, disease duration and gender. Concurrent DMARD therapy and baseline HAQ score were strongly associated with drug response. Specifically, patients on concurrent DMARD therapy demonstrated a significantly greater improvement compared to those without (Coef: 0.50, 95% CI: 0.24, 0.76, $p=1.5x10^{-4}$), whereas higher baseline HAQ scores were significantly associated with smaller improvements (Coef: -0.48, 95% CI: -0.70, -0.26, $p=1.8x10^{-5}$). In addition, females demonstrated a reduced improvement compared to males (Coef: -0.40, 95% CI: -0.68, -0.11, $p = 6.2x10^{-3}$). Baseline age and disease duration, smoking status and concurrent steroid therapy were not associated with drug response at 6 months ($p>0.05$). These findings were expected as associations to the former three factors have been previously reported in the BSR Biologics register, from which the current cohort was recruited (5).

Thus, regression analyses were performed to investigate association of drug response with RF, anti-CCP, SE and *PTPN22* R620W status, adjusting for baseline DAS28, concurrent DMARD therapy, baseline HAQ score and gender (Tables 3). Compared to patients negative for RF, RF-positive patients demonstrated significantly less improvement in their DAS28 values following anti-TNF therapy (Coef: -0.48, 95% CI: -0.87, -0.08, p=0.018) (Table 3). Similarly, patients positive for anti-CCP antibodies demonstrated significantly less improvement in DAS28 compared to anti-CCP negative subjects (Coef: -0.39, 95% CI: -0.71, -0.07, p=0.017) (Table 3). Repeating the analysis after exclusion of patients with a previous exposure to a biologic agent did not alter these conclusions (Table 3).

No association was demonstrated between drug response and either SE or *PTPN22**620W carriage, under any model tested ($p>0.05$) (Table 3).

The effects of RF and anti-CCP antibodies were investigated further by performing multivariate linear regression combining both antibodies. Being positive for both RF and anti-CCP did not better predict response to anti-TNF therapy (RF only: $R^2 = 0.17$, anti-CCP only: $R^2 = 0.17$, RF plus anti-CCP: $R^2 = 0.17$). Furthermore, there was no interaction between these two factors and their association with drug response (RF*anti-CCP: $R^2 = 0.18$, $p = 0.16$). However, as a small proportion of patients were positive for only one antibody, these analyses may be underpowered.

Finally, in order to investigate whether the predictive effects of RF and anti-CCP antibodies were equal for both etanercept and infliximab response, linear regression was performed including the interaction between drug type and autoantibody status. These analyses suggested that, although the effects of both RF and anti-CCP antibodies appeared restricted to infliximab-treated patients, the difference was not statistically different across the two major drug types (Table 4). However,

we had access to serum, but not DNA, samples from a further 240 patients (130 etanercept- and 110 infliximab- treated). When these were included in the analyses, we found that RF, but not anti-CCP, was a significantly greater predictor of response in infliximab rather than etanercept treated patients (difference between drugs- Coef: -0.71, 95% CI: -1.4, -0.02 p=0.05).

Discussion

In the largest such study to date, we have attempted to identify genetic and serological predictors of biologic treatment response. In keeping with previous reports, we have shown that the presence of RF and anti-CCP antibodies is associated with a reduced improvement in the DAS28 score at 6 months, particularly in infliximab-treated patients. No associations were demonstrated between drug response and carriage of risk alleles for either of the 2 known RA susceptibility genes, SE or *PTPN22**620W.

The introduction of anti-TNF biologic agents has transformed the management of RA. However, a substantial proportion of patients still demonstrate partial or no response and there remains a clinical need to develop methods of identifying patients who are more (or less) likely to benefit from such treatments. Predictors of response may include clinical, psychological, serological and genetic factors. Whilst this exploration is still in its infancy, previous studies have suggested that the effect of clinical factors alone is relatively modest (3-5). Hence, in the current study, we have focussed on genetic and serological markers.

There are a number of methodological limitations to the study, which require discussion. Firstly, although the current analyses may inform predictions of how patients receiving anti-TNF therapies will respond to those treatments, the lack of a control group of non-anti-TNF treated

RA patients means that the study cannot inform the debate about whether a patient will respond better to therapy with an anti-TNF rather than a DMARD treatment.

Secondly, response measures were assessed at 6 rather than 3 months, when clinical decisions regarding the continuation of therapy are usually made. Consequently, ~10% of patients had discontinued therapy due to inefficacy prior to the 6 month follow-up and some will have commenced alternative treatment to which they may have responded. Hence, the DAS28 at 6 months may not be a true reflection of the DAS28 when the drug was discontinued. However, this group of patients generally remained non-responders at 6 months despite possibly receiving alternative drugs (mean DAS28 improvement at 6 months: 0.8 compared to 2.7 across remaining cohort). As the study aims to identify predictors of response by 6 months rather than predictors of response only in those who remain on treatment, these patients were included in the analysis although we recognise that this may have resulted in underestimations of observed effects.

Thirdly, as one of the requirements for prescribing anti-TNF agents in the UK includes failure of at least 2 previous DMARDs, the patients recruited have long-standing RA with a mean duration of 14 years. As discussed by Hyrich *et al*, assessing disease activity in such a group can be problematic as current joint swelling and tenderness may be a consequence of structural and irreversible damage caused by previous active disease and not current disease activity (5). Hence, patients with more severe disease as a result of irreversible joint damage may be less likely to respond to treatment. In order to account for this, analyses were repeated adjusting for disease duration, but this did not change the overall conclusions of the study (data not shown). However, as the anti-TNF agents become more commonly prescribed earlier in the disease course, it may be necessary to repeat these analyses.

Fourthly, small numbers of samples exist in some subgroups. In particular, as adalimumab was the most recently approved anti-TNF agent, limited numbers of patients receiving this drug have been collected to date, preventing analyses in this subgroup. As adalimumab is increasingly being prescribed, the continued recruitment of patients should increase the size of this subgroup.

Lastly, the serology has been measured cross-sectionally at the time of sample collection, which may be some time after commencement of treatment. Previous studies have shown that although titres are affected by treatment, status is not (7;25). Hence, in all the analyses, auto-antibody status rather than titre has been used and this may have resulted in loss of power. It should be noted that the proportion of RF positive patients in the current study is higher than that reported previously for the BSRBR cohort as a whole (89% vs. 72%, respectively) (5). This is most likely to be due to differences in data collection methods: the BSRBR study relies on information collected from contributing physicians whereas, for the purposes of the current study, RF was re-measured in all patients for whom a serum sample was available.

Conversely, our study has several advantages over previous investigations. Importantly, the use of the BSRBR to identify suitable patients has meant that the subgroup studied is representative of the BSRBR in its entirety. As, until relatively recently, almost all patients receiving an anti-TNF drug in the UK for RA were included on this register, the cohort studied is likely to reflect the characteristics of anti-TNF-treated patients as a whole, at least in the UK. Furthermore, a wealth of clinical and demographic data had already been collected, creating a well characterised cohort. In addition, this is the largest collection of such patients, to date, allowing robust inferences to be drawn. Finally, the use of the DAS28 measure rather than the EULAR response criteria enhances the power of the study to detect association with genetic predictors of response.

The results of the current study confirm findings of smaller studies in which similar trends between drug response and both baseline RF and anti-CCP antibody titres have been demonstrated (3;4;7). As discussed by Alessandri *et al*, RF and anti-CCP antibodies are independent markers of disease severity for RA (26). Thus the present findings could be interpreted as showing that those patients with the most severe disease are least likely to respond to these therapies. Indeed, there is some evidence to support this hypothesis, as HAQ score, a measure of disability, was also significantly associated with response. However, the association between the autoantibodies and treatment response persisted even after adjustment for the HAQ score suggesting a more complex relationship. Indeed, carriage of SE alleles, previously shown to be associated with disease severity, showed no association with improvement in the DAS28 score.

A number of clinical markers of anti-TNF treatment response have previously been identified including concurrent DMARD therapy, baseline HAQ and gender (3-5). The current study has confirmed that the presence of RF and anti-CCP antibodies can be added to that list but, even when all these factors are combined, only a small proportion of the variance in drug response (R^2=17%) is accounted for. Currently, therefore, a model including these variables will not be useful in the clinical setting. We hypothesize that, in addition to these clinical and serological factors, psychological and genetic factors will play a role and the challenge now is to identify these. No association was observed between treatment response and carriage of the RA susceptibility allele of the *PTPN22* gene and, in keeping with most previous studies, no association of treatment response was observed with SE carriage, a well-established RA severity and susceptibility locus. It is, perhaps, not surprising that genes contributing to disease susceptibility are different to those that determine response to treatment, but large studies of

well-characterised patient cohorts will be required to identify the multiple genes that are likely contribute, each with modest effects, to treatment response.

In summary, the presence of RF or anti-CCP antibodies was associated with a reduced response to anti-TNF drugs as a whole and infliximab, in particular. However, the presence of these antibodies only accounts for a small proportion of the variance in treatment response. It is likely that genetic factors will contribute to determining the response to treatment with these agents but do not include the 2 genes known to confer susceptibility to RA.

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Sandwell General Hospital, West Bromwich (Dr. K A Grindulis, Dr. F Khattak)
Selly Oak Hospital, Birmingham (Dr. Bowman, Prof. C D Buckley, Dr. P Jobanputra, Dr. R W Jubb, Dr. E C Rankin)
St Helens Hospital, St Helens (Dr. V E Abernethy, Dr. J K Dawson, Dr. M Lynch)
The James Cook University Hospital, Middlesbrough (Dr. F Clarke, Dr. J N Fordham, Dr. M J Plant, Dr Tuck)
University Hospital Of North Durham, Durham (Dr. D. Armstrong, Dr. A J Chuck, Dr. S Hailwood)
Whipps Cross University Hospital, London (Dr. S P Donnelly, Dr. D Doyle, Dr. A Hakim, Dr. J G Lanham)

Baseline characteristics	Etanercept	Infliximab	Adalimumab	Combined
Number of cases	278 (43)	296 (46)	68 (11)	642
Age (years)*	57 (11)	58 (11)	59 (12)	57 (11)
Female	223 (80)	228 (77)	51 (75)	502 (78)
Current smokers	56 (20)	51 (17)	6 (9)	113 (18)
Ever smoked	163 (59)	168 (57)	35 (51)	366 (57)
Disease duration (years)*	13 (9)	15 (10)	13 (10)	14 (10)
DAS28*	6.7 (1)	6.7 (1)	6.5 (1)	6.7 (1)
HAQ*	2 (0.6)	2 (0.6)	2 (0.5)	2 (0.6)
Concurrent DMARD(s)	152 (55)	277 (94)	38 (56)	467 (73)
Concurrent steroids	105 (38)	135 (46)	24 (35)	264 (41)
Previous biologic	21 (8)	10 (3)	3 (4)	34 (5)

Table 1. Baseline characteristics
Values are n (%) or mean (SD)*

	Etanercept	Infliximab	Adalimumab	Combined
RF positive	219/241 (91)	189/218 (87)	54/62 (87)	462/521 (89)
Anti-CCP positive	206/241 (86)	177/218 (81)	42/62 (68)	425/521 (82)
SE carriage	184/225 (82)	208/261 (80)	40/49 (82)	432/535 (81)
PTPN22 carriage	78/268 (29)	93/287 (33)	17/64 (27)	188/619 (30)

Table 2. RF, anti-CCP, SE and *PTPN22* status
Values are n of positive/total available (% positive)

Predictor	n* (%)	Mean DAS Score* (SD) Base	Mean DAS Score* (SD) Improvement	Linear regression, Coef. (95% CI) p-value Adjusted 1†	Linear regression, Coef. (95% CI) p-value Adjusted 2‡
RF -ve	59 (11)	6.72 (1)	3.03 (1.7)	ref	ref
RF +ve	462 (89)	6.59 (1)	2.43 (1.5)	-0.48 (-0.87, -0.08) p=0.02	-0.48 (-0.89, -0.07) p=0.02
Anti-CCP -ve	96 (18)	6.61 (1)	2.90 (1.6)	ref	ref
Anti-CCP +ve	425 (82)	6.61 (1)	2.40 (1.5)	-0.39 (-0.71, -0.07) p=0.02	-0.39 (-0.72, -0.06) p=0.02
SE -ve	103 (19)	6.65 (1)	2.38 (1.5)	ref	ref
SE +ve	432 (81)	6.71 (1)	2.49 (1.5)	0.07 (-0.25, 0.39) p=0.68	0.06 (-0.26, 0.39) p=0.70
PTPN22 -ve	431 (70)	6.67 (1)	2.51 (1.6)	ref	ref
PTPN22 +ve	188 (30)	6.72 (1)	2.48 (1.4)	-0.11 (-0.36, 0.15) p=0.41	-0.13 (-0.39, 0.13) p=0.34

Table 3. Linear regression for RF, anti-CCP, SE and *PTPN22*
*Figures represent those across complete subgroup of 642 patients. †Initial analyses were performed across the entire cohort, djusting for baseline DAS28, HAQ, concurrent DMARD therapy and gender. ‡Subsequent analyses excluded patients with previous exposure to a biologic agent. SD = standard deviation, Coef. = coefficient, CI = confidence interval, ref = reference group.

Predictor	n* (%)	Mean DAS Score* (SD)		Linear regression Coef. (95% CI) p-value	
		Base	Improvement	Adjusted†	Difference between drugs
etanercept					
RF –ve	22 (9)	6.63 (1)	2.69 (1.8)	ref	ref
RF +ve	219 (91)	6.63 (1)	2.43 (1.5)	-0.25 (-0.89, 0.39) p=0.44	
infliximab					-0.58 (-1.4, 0.03) p=0.18
RF –ve	29 (13)	6.79 (1)	3.34 (1.6)	ref	
RF +ve	189 (87)	6.60 (1)	2.34 (1.6)	-0.83 (-1.40, -0.27) p=0.004	
etanercept					
Anti-CCP -ve	35 (15)	6.62 (1)	2.72 (1.4)	ref	ref
Anti-CCP +ve	206 (85)	6.64 (1)	2.40 (1.5)	-0.26 (-0.77, 0.26) p=0.33	
infliximab					-0.41 (-1.1, 0.3) p=0.26
Anti-CCP -ve	41 (19)	6.62 (1)	3.07 (1.7)	ref	
Anti-CCP +ve	177 (81)	6.63 (1)	2.33 (1.6)	-0.67 (-1.16, -0.18) p=0.007	

Table 4. Linear regression of RF and anti-CCP, stratifying for anti-TNF agents

*Figures represent those across complete subgroup of 642 patients. †Analyses adjusted for baseline DAS28, HAQ, concurrent DMARD therapy and gender. SD = standard deviation, Coef. = coefficient, CI = confidence interval, ref = reference group.