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# INFLIXIMAB IN COMBINATION WITH METHOTREXATE IN ACTIVE ANKYLOSING SPONDYLITIS:

## A clinical and imaging study

Helena Marzo-Ortega, Dennis Mc Gonagle, Stephen Jarrett, Glenn Haugeberg, Elizabeth Hensor, Philip O'Connor, Ai Lyn Tan, Philip G Conaghan, Adam Greenstein and Paul Emery

(1) Helena Marzo-Ortega MRCP, Specialist Registrar
(1, 2) Dennis McGonagle PhD FRCPI, Professor of Investigative Rheumatology
(1)Stephen Jarrett MRCP, Specialist Registrar
(3)Glenn Haugeberg MD PhD, Consultant Rheumatologist
(1) Elizabeth Hensor BSc PhD, Data Analyst
(4) Philip O'Connor FRCR, Consultant Radiologist
(1) Ai Lyn Tan MRCP, Research Fellow
(1) Philip G Conaghan MBBS FRACP, Senior Lecturer and Honorary Consultant Rheumatologist
(1) Adam Greenstein MRCP, Specialist Registrar
(1) Paul Emery MA MB FRCP, arc Professor of Rheumatology

(1) Academic Unit of Musculoskeletal Disease, Department of Rheumatology and Rehabilitation. 1st Floor, Leeds General Infirmary. Great George Street, Leeds LS1 3EX, UK
(2) Department of Rheumatology. Calderdale General Hospital, Salterhebble, Halifax HX3 0PW, UK
(3) Department of Rheumatology, Vest-Agder Hospital. Serviceboks 416. N-4604 Kristiansand Norway.
(4) Department of Radiology, Leeds General Infirmary. Leeds LS1 3EX, UK

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Address for correspondence:
Professor Paul Emery
Academic Unit of Musculoskeletal Disease
Department of Rheumatology
1st Floor, Old Nurses Home
Leeds General Infirmary
Great George Street
Leeds LS1 3EX

T: 00-44-113-3925068
Fax: 00-44-113-3922896
e-mail: p.emery@leeds.ac.uk
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**Background and Aims**. Infliximab monotherapy is an effective treatment for ankylosing spondylitis (AS) but in RA its efficacy is increased and toxicity decreased when used in combination with methotrexate. The aims of this study were to examine the efficacy and safety of infliximab combined with methotrexate versus methotrexate alone in the treatment of AS. Magnetic resonance imaging (MRI) and bone densitometry (DXA) were performed to monitor its impact on bone.
**Methods**. In this single centre study 42 subjects with active AS were treated with methotrexate and were randomly assigned to receive 5 infusions of either 5mg/kg infliximab or placebo over a 30 week period with a 2:1 randomisation in favour of the combination arm. The primary outcome was improvement in disease activity as shown by the BASDAI at week 30. MRI was used to assess the effect of therapies on sacroiliac and spinal enthesitis/osteitis and DXA was used to monitor bone mineral density (BMD).
**Results.** Both therapeutic agents were well tolerated with no dropouts due to adverse events. A significantly greater improvement in mean BASDAI score was seen in the infliximab arm at week 10 ($p=0.017$) compared to the placebo arm, but this was not maintained by week 30 ($p=0.195$), eight weeks after the last infusion (week 22) at which stage disease flares were reported by some subjects. On MRI, the mean number of lesions that resolved per subject from week 0 to week 30 was significantly greater in the combination group than in the methotrexate monotherapy group ($p=0.016$).
**Conclusions.** Infliximab in combination with methotrexate was a safe and efficacious therapy in AS over a six-month period and was associated with significant regression of MRI determined enthesitis/osteitis. However disease flares were reported 8 weeks after last infusion indicating that the addition of methotrexate failed to extend the infliximab dosing interval.

Ankylosing spondylitis (AS) is a chronic inflammatory disorder that predominantly affects young adults. Conventional therapeutic options for AS, unlike rheumatoid arthritis (RA) fail to prevent disease progression with a substantial proportion of subjects (40%) eventually developing severe spinal restriction (1). Furthermore, AS carries substantial morbidity and reduced quality of life similar to that seen in RA (2). The short term management of AS has been transformed by the introduction of tumour necrosis factor (TNF) α blockers with data from double-blind and open label studies using the monoclonal antibody infliximab or the Fc-TNFR fusion protein etanercept showing efficacy (3, 4, 5) similar to that seen in RA subjects.
In RA, methotrexate is used as the anchor drug for combination therapy both with other disease modifying anti-rheumatic drugs (DMARDs) and with biologic therapies (6). However, there are no data demonstrating the efficacy of methotrexate as monotherapy in AS, apart from some preliminary results in small open label trials (7, 8). Although infliximab generally is well tolerated, hypersensitivity and infusion related reactions have been reported with its use. It has been postulated that these effects may in part be related to the development of human antibodies against the chimeric part of the molecule (HACA) and that concurrent use of immunosupressant drugs such as methotrexate may decrease the development of these (9) and hence reduce some of the side effects associated with its use. Furthermore, there are data suggesting that the combination of infliximab and methotrexate may be more effective that either drug alone (10) which may be partly explained by an increase in drug levels but also by a possible synergistic effect on disease pathogenesis. It is unknown whether the same benefits from using methotrexate or other immunosuppressant drugs would accrue in subjects with AS, where disease flares are known to occur six weeks following therapy.

Magnetic resonance imaging (MRI) is a sensitive imaging tool that allows for excellent visualization and multiplanar assessment soft tissues and bone and can identify acute and chronic lesions in AS 11). Recently, the role of MRI in the diagnosis and monitoring of sacroiliac and spinal disease activity in AS has been explored by different groups (12), and although more validation work needs to be done, it is rapidly becoming the imaging method of choice in AS. Likewise, DXA is a sensitive method of quantifying bone mineral density (BMD). Importantly osteoporosis is an early feature of active AS (13). The aims of this study were therefore to assess efficacy, safety and duration of response to the combination of infliximab with methotrexate in AS and to assess whether the addition of methotrexate could prolong response to infliximab therapy.

## Methods

### Study design and randomisation

This study was designed as a 30-week, single centre, randomised, double blind placebo controlled trial and had the approval of the Local Research Ethics Committee. All subjects gave informed written consent. A randomisation list was generated by a statistician (unconnected with the final analysis of results) on a 2:1 basis, with 2 thirds of the subjects being included on the infliximab group and 1 third on the placebo group. Study participants, clinical observer and metrologists were blinded to the randomisation code that was kept in the hospital pharmacy.

### Study drugs

Infusions of infliximab (5mg/kg in 250 ml 0.9% NaCl) or placebo were prepared by the hospital pharmacy under aseptic conditions. Infusion regime was weeks 0, 2, 6, 14

and 22. In addition all subjects were provided at week 0 with a prescription for oral methotrexate at a dose of 7.5 mg with folic acid cover (5 mg twice a week), which would be eventually increased to 10 mg per week.

**Subjects**

Subjects eligible for the study were recruited from specialist rheumatology clinics in the Yorkshire region and needed to fulfil the modified New York criteria for AS (14), be older than 18 years of age and have active spinal involvement. This was defined as persistent inflammatory back pain (defined as 3 cm or more on a 10 cm visual analogue scale [VAS]) and a raised inflammatory response in serum as shown by a C-reactive protein value of more than 10 mg/L despite treatment with conventional agents such as optimal dosage of non-steroidal anti-inflammatory drugs (NSAIDs) or DMARDs. Exclusion criteria included any history of tuberculosis, active infection, demyelinating disease, previous lymphoproliferative or malignant disorder, pregnancy, breastfeeding or uncontrolled concomitant disease in the opinion of the investigator. Subjects who had received an investigational drug within 3 months of the beginning of the study were excluded.

Subjects were allowed to continue NSAIDs and/or oral corticosteroids, provided the dose was unaltered throughout the study period. Other DMARDs were stopped at least 4 weeks before the baseline visit. No intra-articular or intra-muscular injections of corticosteroids were allowed during the study. If these were required because of unacceptably high level of disease activity, subjects could be dropped out of the study at the investigator's discretion.

**Assessments of efficacy and outcome**

Subjects were seen for clinical evaluation at baseline, weeks 4, 10 and 30. The following variables were evaluated: Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) (15), Bath Ankylosing Spondylitis Functional Index (BASFI) (16), 10 cm visual analogue scales (VAS) to measure spinal pain during the day and night, as well as the physician global assessment of disease activity and duration of early morning stiffness (minutes). Item number 4 of the BASDAI, considered to represent a VAS for enthesopathy, was analysed independently. In addition a quality of life instrument, the ASQoL (Ankylosing Spondylitis Quality of Life questionnaire) (17) was used. Laboratory tests performed included the measurement of inflammatory markers such as the C-reactive protein, complete blood count, liver function tests and levels of antinuclear antibodies (ANA). HLA-B27 type was performed in all cases at baseline.

The primary outcome was evaluation of change in BASDAI score at weeks 4, 10 and 30. Secondary outcomes included comparison of the proportions of subjects in each arm achieving response criteria proposed by the ASAS group (18). This is presented as ASAS 20, defined as a 20% improvement seen of at least 10 units (scale 0-100) in at least 3 of the following 4 domains: subject's global assessment, pain, function (as represented by BASFI), and inflammation (represented by morning stiffness measures as described in BASDAI), in the absence of deterioration in the remaining domain. In addition, the recently defined ASAS response for biologics criteria (19) was analyzed. According to this, a subject was deemed to be a responder if they satisfied the following criteria: BASDAI: 50% relative change or absolute change of 2 (scale 0-10) and expert opinion.

**Imaging**

**Magnetic Resonance Imaging (MRI):** Scans of the sacroiliac joints (SIJ) and lumbar spine were performed at 0 and 30 weeks using a commercially available 1.5 T Philips Gyroscan ACS NT (Philips Medical Systems, Best, The Netherlands).
The following sequences were used: T1-weighted turbo spin-echo, T2-weighted SPIR (fat suppressed [FS]) coronal oblique sequences and T1 FFE SPIR Post Gadolinium (Gad-DTPA) (vol 1.5 mm) of the SIJ as well as T2 SPIR sagittal sequence of the lumbar spine. MR parameters were as follows: a spin-echo sequence with T1-weighted images (repetition time [TR] 908 ms, time to echo [TE] 14 ms, matrix 192/256, field of view [FOV] 320 mm, slice thickness 4.0 mm, slice gap 0.3 mm, number of signals averaged [NSA] 3, and acquisition time 2:56 m) was used for the SIJ. T2 TSE/FS acquisition parameters were as follows: TR 2125 ms, TE 120 ms echo train length (ETL), matrix 252/512, FOV 320 mm, slice thickness 4.0 mm, slice gap 0.4 mm, NSA 3 and acquisition time 2:54 m for the SIJ and a TR 1327 ms, TE 120, FOV 320, slice thickness 4.0/0.4, matrix 247/512 with an acquisition time of 2:29 m for the spine. Active disease was defined on T2 FS images as bone oedema (identified by high or intermediate marrow signal) and/or soft tissue oedema (high signal in the extracapsular connective tissues) as previously described (20).
MRI scoring: MRI scans were anonymized and assigned random numbers by an independent assessor. Two experienced observers blinded to the subjects' clinical characteristics and to time sequence scored paired scans using a scoring system previously described where sites of spinal/sacroiliac changes of active enthesitis and osteitis were evaluated (20). Where there was disagreement between the two observers consensus was established. As previously reported (20), the SIJ was divided into four quadrants for assessment: right upper, left upper, right lower and left lower. Each quadrant was subdivided into ilial and sacral aspect. Lesions were scored using a semi-quantitative scale (0-3). In the spine, lesions were classified as present or absent within the vertebral bodies, the spinous processes, facetal joints or in the paraspinal soft tissues. A total count of lesions per spinal area per subject was performed. Degree of change between baseline and follow- up scans was assessed using a semiquantitative scale (resolution, improvement, no change, new lesions). The intra-rater and inter-rater reliability of this system for acute oedematous lesions using ICC statistics were: intra-rater= (range) 0.64 -1.00 for the sacro-iliac joints and 0.6-0.77 for the spine; inter-rater= (range) 0.67-0.85, and 0.80-0.93 for the sacro-iliac joints and the spine respectively.

In addition, to assess the effect of both therapies on bone mass, all subjects underwent dual X-ray absorptiometry (**DXA)** examination to measure bone mineral density (BMD) at the hip (femoral neck and total hip) and spine (L2-L4) at baseline and 30 weeks. All scans were performed by one technician using the same DXA equipment (Lunar Expert, Madison, Wisconsin). For hip BMD analyses, mean values from left and right hip. Short-term in-vivo precision was 1.43% at total hip, 2.89% at femoral neck and 2.42% at spine. The long-term spine phantom precision for the whole study period was 0.80%.

**Statistical analysis**

The study was designed with 90 % power to detect a response of up to 30% on the placebo group and 80% in the infliximab group, using Holm-corrected ANCOVA analysis at the $\alpha$=0.017 level, based on preliminary results from open label studies (21,22) available at the time of the study design. A 2:1 randomization was applied with twice as many subjects on the treatment group, as it was anticipated that the efficacy of methotrexate would be comparable to that of placebo reported in previous

trials. This translated into a target sample of 42 subjects to allow for the withdrawal of up to 2 subjects per treatment group. An intention-to-treat analysis was performed and where a subject withdrew before study completion then a last observation carried forward method was used for missing values. ANCOVA was used to compare the degree of change in BASDAI from baseline in the two treatment groups taking baseline BASDAI as a covariate. Paired t-tests were used to assess whether within each treatment group there was a significant reduction in BASDAI from baseline. Non-parametric statistics were applied to all secondary outcome measures, with the exception of the MRI and DXA results, on which ANCOVA and t-tests were performed respectively. Fisher's Exact tests were used to compare proportions of responders between groups, Mann-Whitney U tests were performed to test for differences between groups at baseline, and to compare change scores between groups at weeks 10 and 30. Wilcoxon signed rank tests were used to test for changes within groups from baseline to week 30. Corrections for multiple comparisons were made separately for primary and secondary outcome measures, within families of statistical tests, following the Holm technique (23). Critical p for testing at the $\alpha$=0.05 level was therefore set at p=0.017 for ANCOVA, p=0.008 for paired t-tests (primary outcome) and Fisher's exact tests, p=0.017 for paired t-tests (secondary outcome), p=0.017 for independent t-tests, p=0.005 for Wilcoxon signed rank tests and p=0.003 for Mann-Whitney U tests.

## Results

### Study population

Forty two subjects were randomized, 28 to infliximab and 14 to placebo. Both groups started methotrexate 24 hours after the first infusion. One subject in the infliximab group withdrew consent after randomisation which left 41 subjects for initial analysis. Another 6 subjects withdrew from the study before week 30, of these, 4 subjects on the placebo group dropped out before week 4 due to lack of efficacy. Two more subjects, one from each group, withdrew before week 10 for personal reasons (Figure 1). The remaining 35 subjects, 24 on infliximab (86%) and 11 on placebo (79%) continued in the study until week 30. The demographics and characteristics of the subjects in both groups are summarized in Table 1.

### Efficacy results

The intention-to-treat primary analysis showed that subjects given infliximab in combination with methotrexate showed a greater reduction in BASDAI score at weeks 4, 10 and 30 (mean change –2.0, -3.1 and –1.9 respectively) compared to the subjects treated with methotrexate alone (mean change –0.6, -1.4 and –0.8). However this response only achieved statistical significance at week 10 (p=0.017) and was not maintained at week 30 (p=0.195), probably reflecting the fact that a number of subjects in the infliximab group reported a flare by week 6 post last study infusion. A similar response was also observed in objective parameters of disease activity such as the CRP the improvement in which was significantly greater in the combination group by week 10 (p<0.001) but not significantly different between treatment groups by week 30 (p=0.017, Table 2). Indeed, further subanalysis of the CRP response at the interim visits (weeks 14 and 22) shows a marked deterioration on CRP values by week 22 (mean % change 69.3) on the subjects (n=5) that subjectively reported a flare of disease in between study visits (Figure 2). Looking at the ASAS20 response, no statistically significant differences were observed between the groups at any endpoint (46% vs 21% at week 4; p=0.180; 71% vs 28% at week 10, p=0.019; and 50% vs 21% at week 30, p=0.102). When analysing the composite ASAS response criteria for biologics, a significantly greater proportion (50%) of subjects in the infliximab group

achieved ASAS response at week 4 against 7% in the placebo group (p=0.007) although this response was not maintained at weeks 10 (67% vs 35%; p=0.096) or 30 (53% vs 21%; p=0.057) [Figure 3]. On detailed analysis of the groups independently it was clear that the biggest benefit occurred in the combination group (Table 3) where the level of response was equivalent to previously published results (4).

**MRI results:** Eight subjects were unable to undergo MRI scanning due to severe postural abnormalities. In addition, a further 6 subjects either dropped out during the study or did not attend for their follow up scan. In total, pre and post therapy MRI scans from 19 subjects in the combination group and 9 subjects in the methotrexate monotherapy group were available for analysis.
Overall, 85% of the subjects had lesions consistent with active disease (n=17 in the combination group, n=7 in the methotrexate monotherapy group). At baseline, a total of 97 lesions were seen in the combination group (72 lesions in the lumbar spine and 25 lesions in the SIJs) and 107 in the methotrexate monotherapy group (67 in the lumbar spine, 40 in the SIJs). Although an improvement was seen in both groups by week 30, the mean number of lesions per subject that had resolved completely by week 30 was significantly greater in the combination group (mean 4.7, 95% C.I. 3.3 to 6.1) than in the methotrexate monotherapy group (mean 1.4, 95% C.I. -0.8 to 3.5, p=0.016). There was a trend towards the mean number of lesions per subject that remained unchanged following treatment being greater in the methotrexate monotherapy group (mean 5.5, 95% C.I. 3.3 to 7.7) than in the combination therapy group (mean 2.6, 95% C.I. 1.1 to 4.1), however following correction for multiple comparisons this difference was not statistically significant (p=0.038). The mean numbers of new lesions per subject did not differ significantly between treatment groups (methotrexate mean 0.9, 95% C.I. -0.1 to 2.0, combination mean 0.9, 95% C.I. -0.2 to 1.6, p=0.965). A total of 19 new lesions appeared in the combination group (15 in the spine and 4 in the SIJs), all in subjects that reported flares before week 30. In the methotrexate monotherapy group 7 new lesions appeared (6 in the spine, 1 in the SIJs).
Combining both treatment groups, a significant association was identified between the level of improvement in BASDAI score and the numbers of lesions that resolved per subject during treatment (Spearman rank correlation, p=0.04, see Figure 4).

**DXA results:** At 30 weeks in the combination group, hip and lumbar spine BMD data were available in 23 and 24 subjects respectively. In the methotrexate monotherapy group, hip BMD data was available in 8 subjects and lumbar spine BMD data was available in 10 subjects. As shown in Figure 5 a statistically significant increase in total hip BMD was seen in the infliximab treated group (+1.9%, paired t-test p=0.004), with trends towards increases in femoral neck (+2.5%, p=0.03) and spine (+3.6%, p=0.02) BMD, whereas no significant change was seen in the methotrexate monotherapy group at either the femoral neck (–1.3%, p=0.47), total hip (+0.1, p=0.90) or spine (–1.4%, p=0.50). Although there was a trend towards improved BMD in the combination group, comparison between the groups failed to show a significant difference (independent t-test p-values ranged from 0.06 to 0.14).

**Safety data**
Both study drugs were well tolerated with the majority of side effects being mild to moderate in severity (Table 4). Two subjects in the combination group developed a mild hypersensitivity reaction after the 1st infusion that settled spontaneously in the

first subject and was controlled with regular hydrocortisone cover in the second case. No severe adverse events were seen in either group.

**ANA detection**

Four subjects in the combination group were ANA positive at baseline in a weak titre (1/40: n=3 and 1/80: n=1). No subjects in the methotrexate monotherapy group were ANA positive at baseline. At 30 weeks, a total of 11 (39%) subjects were ANA positive in the combination group. Of the 4 subjects that were initially ANA positive, one subject (ANA 1/40 titre at baseline) was negative at week 30. The remaining 3 remained positive with a higher titre (one 1/160, two 1/640, all homogeneous pattern). A further 8 subjects (28%) in the combination group became ANA positive at week 30 with mildly elevated titres. None of the subjects developed clinical symptomatology suggestive of a connective tissue disorder. No ANA seroconversion was seen in the methotrexate monotherapy group by week 30.

**Discussion**

Tumour necrosis factor-α blockade with infliximab is an important therapeutic advance for subjects with RA and AS. In RA, infliximab used in conjunction with methotrexate results in superior safety and efficacy but this has not been established in AS. This is the first study to look at the efficacy and safety of the combination of infliximab and methotrexate in AS. These results show that the combination regime was well tolerated with no discontinuations due to side effects over a 6 month period. This regime was highly efficacious at 10 weeks reflecting the immediate improvement known to occur with infliximab but this effect was not significant at 30 weeks. This reflects the disease flare that was reported by some of the subjects 8 weeks following the last infusion.

Recent reports of monotherapy with infliximab in AS showed a higher incidence of severe adverse events leading to discontinuation of therapy (24). In this study the combination of infliximab and methotexate was well tolerated with no serious side effects. In addition, although the prevalence of ANA seroconversion was similar to that reported in other studies, this did not appear to be clinically relevant at 30 weeks. Again these results contrast with those found by other authors (25) and although inter-laboratory variability on ANA testing should always be considered (26), we believe that this effect may be due to the concomitant use of methotrexate.

The extended infusion regime interval of 8 weeks was chosen because we postulated that concomitant administration of methotrexate would enhance the duration of response. Accordingly, the primary outcome was set at week 30, eight weeks after the last infusion was given (week 22). Other studies have reported impressive clinical response at 12 weeks (comparable to the 10 week efficacy assessments in the present study). However, a number of subjects in our study reported a return of symptomatology within 6 weeks of the last infusion accounting for the apparent lack of efficacy at week 30. In clinical practice this would be overcome by shorter infusion schedules and indeed new guidelines suggest an appropriate infusion interval of 6 weeks (19).

The level of response seen in the infliximab treated group when using clinical parameters such as the BASDAI is comparable to previous reports. These results also

show a small degree of improvement in the monotherapy group treated with methotrexate which was comparable to the placebo arm of other studies (4). However, there was an improvement in the MRI score in the methotrexate group compared to deterioration in historical studies with placebo controls (27). This may mean that methotrexate may have a favourable effect as suggested in small uncontrolled studies (7, 8) and needs to be confirmed in larger studies in which higher therapeutic doses of methotrexate are achieved.

As shown by imaging methods, the majority of MRI determined lesions in the infliximab treated group improved by week 30 but interestingly, new regions of enthesitis/osteitis were evident in those subjects who reported a disease flare prior to the last clinical assessment. In the DXA analysis a clear improvement was observed in the combination treated arm confirming previous observations from our group that adequate suppression of inflammation leads to improvement of bone mass in subjects with active disease (28). Therefore biological therapy suppresses the primary spinal abnormalities but also may reverse secondary abnormalities such as osteoporosis. These imaging findings have important implications for the long-term management of these subjects; as osteoporosis is a known complication of active AS and may occur early in the disease process (13, 29).

In all, a number of limitations in the study set up need to be taken into account when interpreting these results. Methotrexate was chosen because of previous reports in RA suggesting an immunogenic effect that can be achieved with only a small dose. Because of the lack of data suggesting a therapeutic effect in AS, no higher doses were sought. In addition the drop out rate from the study was unexpectedly high making result interpretation difficult due to wide differences in standard deviations.

In AS, distinguishing between spinal inflammation and spinal fusion, both of which lead to loss of function, is problematic. In this study where conventional, clinically based outcomes and imaging determined outcomes were used, the latter were found to be very sensitive for assessing therapy. Whilst suggesting efficacy for both treatment arms, the response was considerably better for the infliximab arm, confirming that MRI is a valid tool to assess disease activity in AS. In addition, this study illustrates a scoring method that is reliable and has successfully shown sensitivity to change over a six month period after treatment with different biologic agents (17). As is the experience in other units, this scoring method was developed by the joint effort of rheumatologists and radiologists confirming the need for a close liaison between related specialties in the search for better tools to diagnose and assess disease activity.

In conclusion this study confirmed that infliximab in combination with methotrexate was safe in AS but the addition of methotrexate did not sustain response for eight weeks.

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**Figure 6. Magnetic resonance images of spine and sacro-iliac joints pre and post therapy**. **Fig 6A.** T2-weighted fat-suppressed (FS) sagittal sequence of the lumbar spine of a patient showing acute Romanus lesions (thin white arrows) at the anterior inferior aspects of L1 and L3, and anterior superior aspects of L2 and L4 vertebral bodies. **Fig 6B.** Illustrates complete resolution of the lesions after treatment with infliximab and methotrexate. **Fig 6C.** T2-weighted FS coronal oblique image of the sacroiliac joint of another patient showing active sacroiliitis (thick white arrow) which has improved markedly after treatment with infliximab and methotrexate (**Fig 6D**).

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## Tables

Table 1. Baseline characteristics of the study patients. Values presented are median (range) unless otherwise stated.

| | **Infliximab + MTX group (n=28 )** | **Placebo + MTX group (n=14 )** |
|---|---|---|
| Male:Female | 23:5 | 11:3 |
| Age (years, mean) | 41 (28-74) | 39 (30-56) |
| Disease duration (years) | 8 (0-41) | 10 (0-35) |
| HLA-B27 positive (%) | 96 | 86 |
| BASDAI | 6.9 (2.11-9.26) | 6.4 (3-10) |
| BASFI | 6.7 (1.9-9.63) | 6.0 (3.8-10) |
| VAS (mm):<br>- Pain day<br>- Pain night<br>- Enthesopathy | <br>57.5 (17-96)<br>63.5 (11-100)<br>75 (0-100) | <br>66 (24-100)<br>76.5 (33-100)<br>73 (9-100) |
| EMS (min) | 60 (15-120) | 75 (10-120) |
| C-reactive protein (mg/L) | 30.5 (10-153) | 30 (13-60) |
| ASQoL | 14 (2-18) | 13.5 (8-18) |
| Concomitant medication (number of patients, %):<br>- NSAIDS<br>- Oral corticosteroids<br>- DMARDs | <br>25 (85.7)<br>5 (17.8)<br>10 (35.7) | <br>12 (85.7)<br>3 (25)<br>4 (28.5) |

No statistically significant differences were found between treatment groups in any of the baseline characteristics using non-parametric tests (Mann-Whitney U test). BASDAI: Bath Ankylosing Spondylitis Disease Activity Index 0-10, BASFI: Bath Ankylosing Spondylitis Functional Index 0-10, ASQoL: Ankylosing Spondylitis Quality of Life index, VAS: visual analogue scales, EMS: early morning stiffness.

Table 2. ITT analysis of clinical outcomes at all endpoints. Values are given median (range).

| | **Infliximab + methotrexate group (n=28)** | | | | **Placebo + methotrexate group (n=14)** | | | | ***INF vs Plac P=*** |
|---|---|---|---|---|---|---|---|---|---|
| ***Variable*** | ***Baseline*** | ***Week 4*** | ***Week 10*** | ***Week 30*** | ***Baseline*** | ***Week 4*** | ***Week 10*** | ***Week 30*** | ***Week 30*** |
| BASFI | 6.68 (1.90-9.63) | 5.61 (0.32-9.60) | 4.96 (0.32-8.84) | 5.04 (0.61-9.14)* | 6.00 (3.84-10) | 4.90 (0.23-9.66) | 6.10 (0.89-9.59) | 5.68 (2.69-9.59) | 0.196 |
| EMS | 60 (15-120) | 45 (0-120) | 15 (0-120) | 30 (0-120) | 75 (10-120) | 37.5 (0-120) | 45 (0-120) | 60 (5-120) | 0.298 |
| VAS spinal pain during day | 57.5 (17-96) | 28.5 (0-100) | 20.5 (0-100) | 30 (0-100) | 66 (24-100) | 60 (6-100) | 55 (6-98) | 58.5 (17-98) | 0.043 |
| VAS spinal pain night | 63.5 (11-100) | 21 (0-100) | 11 (0-93) | 22 (0-93)* | 76.5 (33-100) | 65 (12-100) | 63.5 (6-97) | 58.5 (22-97) | 0.235 |
| VAS enthesitis | 75 (0-100) | 30.5 (0-83) | 16 (0-100) | 29.5 (0-100)* | 73 (9-100) | 62 (8-100) | 51 (11-98) | 46 (11-98) | 0.488 |
| Physician DAS | 63.5 (36-96) | 24.5 (4-80) | 19.5 (2-54) | 23.5 (0-65)* | 62 (29-98) | 46.5 (8-78) | 48.5 (12-78) | 57.5 (27-92) | 0.001** |
| ASQoL | 14 (2-18) | 10.5 (0-18) | 7.5 (0-17) | 8.5 (0-18)* | 13.5 (8-18) | 11.5 (0-18) | 14 (2-18) | 14.5 (5-18) | 0.144 |
| CRP | 30.5 (10-153) | 6 (0-104) | 5.5 (0-50) | 8.5 (0-79)* | 30 (13-60) | 22 (10-52) | 21 (7-92) | 26 (7-56) | 0.017 |

* $P < 0.005$ - Wilcoxon signed rank test comparing baseline data to week 30 within groups.
** $P < 0.003$ - Mann-Whitney U test comparing change scores (week 30 minus baseline) between groups.

Table 3. BASDAI response within the groups (*statistically significant difference).

| Treatment | Baseline | Week 4 | | | Week 10 | | | Week 30 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean (SD) | Mean (SD) | Mean Diff (SD) | P = | Mean (SD) | Mean Diff (SD) | P = | Mean (SD) | Mean Diff (SD) | P = |
| **Infliximab** | 6.45 (1.87) | 4.48 (2.58) | 1.97 (2.22) | <0.001* | 3.34 (2.56) | 3.11 (2.23) | <0.001* | 4.60 (2.85) | 1.85 (2.84) | 0.002* |
| **Placebo** | 6.57 (2.05) | 5.92 (2.34) | 0.65 (1.47) | 0.124 | 5.19 (2.52) | 1.38 (2.11) | 0.030 | 5.74 (2.34) | 0.84 (1.80) | 0.106 |

Table 4. Drug related adverse events.

| | **Infliximab + MTX group (n=28)** | **Placebo + MTX group (n=14)** |
|---|---|---|
| Minor Infections: | | |
| -Upper respiratory tract infections | 2 | 2 |
| -Sinusitis | 1 | 0 |
| -Sore throat | 1 | 0 |
| -Oral thrush | 1* | 0 |
| Transient elevation in transaminases | 2* | 0 |
| Chest infection | 1 | 0 |
| Shingles | 1 | 0 |
| Pruritus | 1 | 0 |
| Urticarial rash | 1 | 0 |
| Iritis | 1 | 0 |
| Infusion reactions | 1 | 0 |
| **TOTAL** | **13 (46%)** | **2 (1.4%)** |

Values are number of patients experiencing at least 1 episode of adverse event per category. * Both events were felt to be more related to methotrexate than infliximab. Oral thrush settled with increase in folic and topical therapy. In one patient transaminases settled after transient reduction in MTX dose.

## Figures

Figure 1. Randomisation, reasons for treatment discontinuation and numbers of patients who completed the 30 week study period.

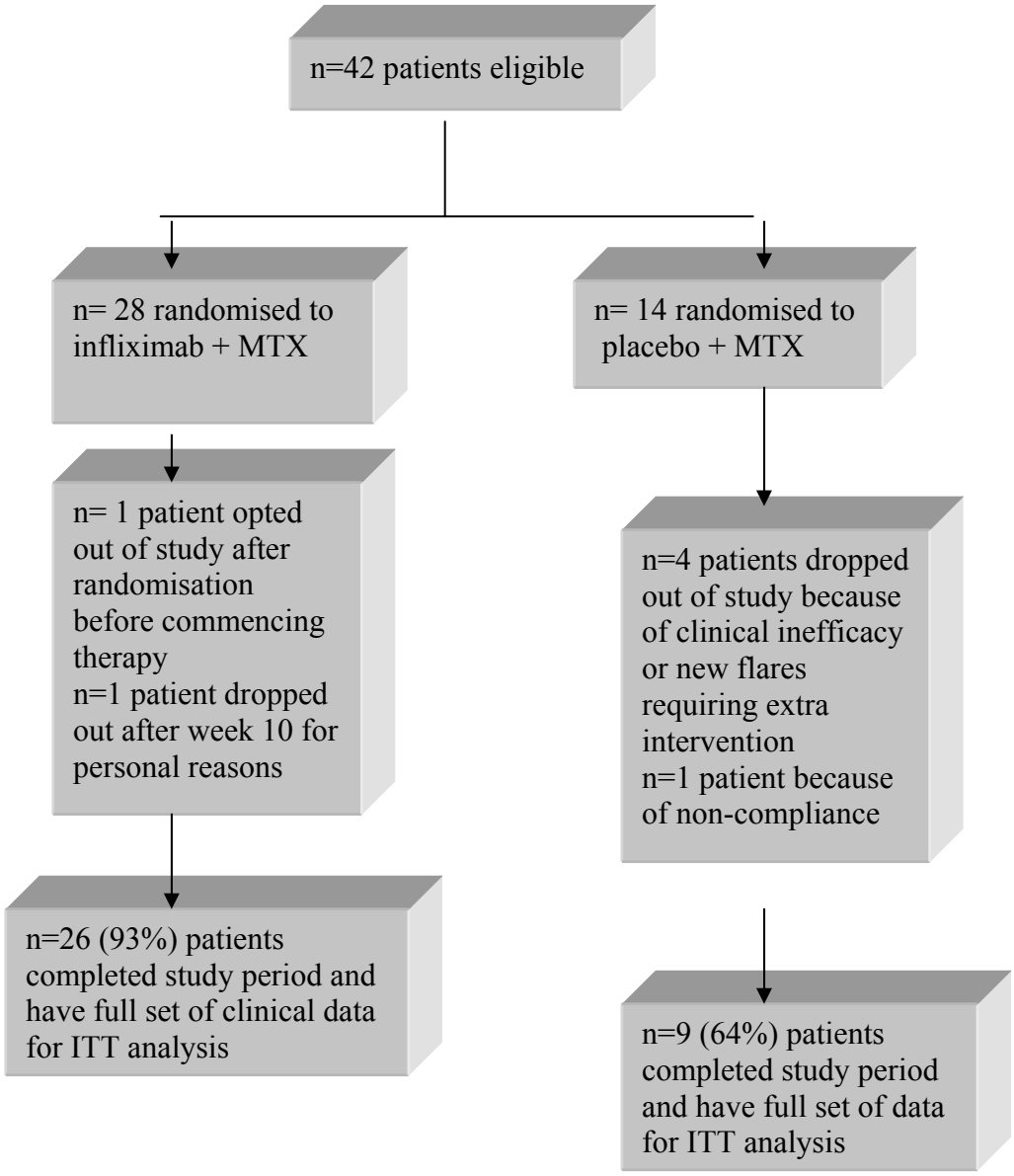


Figure 2. Analysis of CRP results at all study visits looking at the subset of patients in the infliximab treated group that reported a flare of disease in the interim visits.

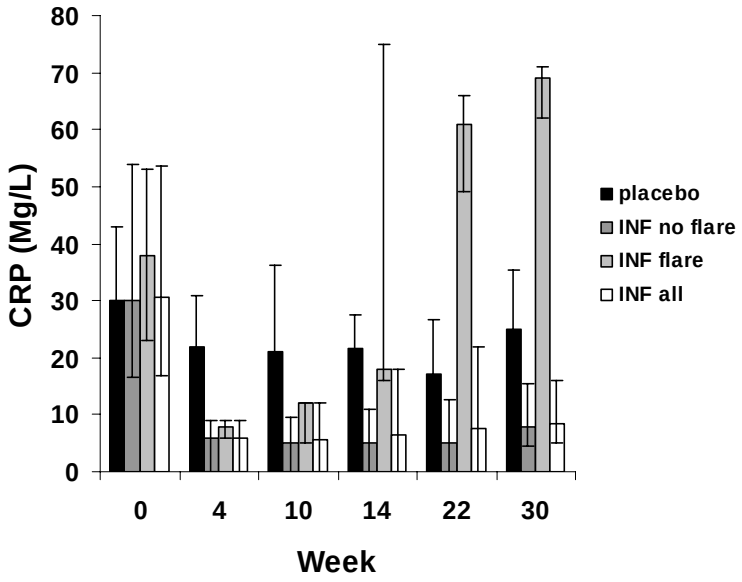
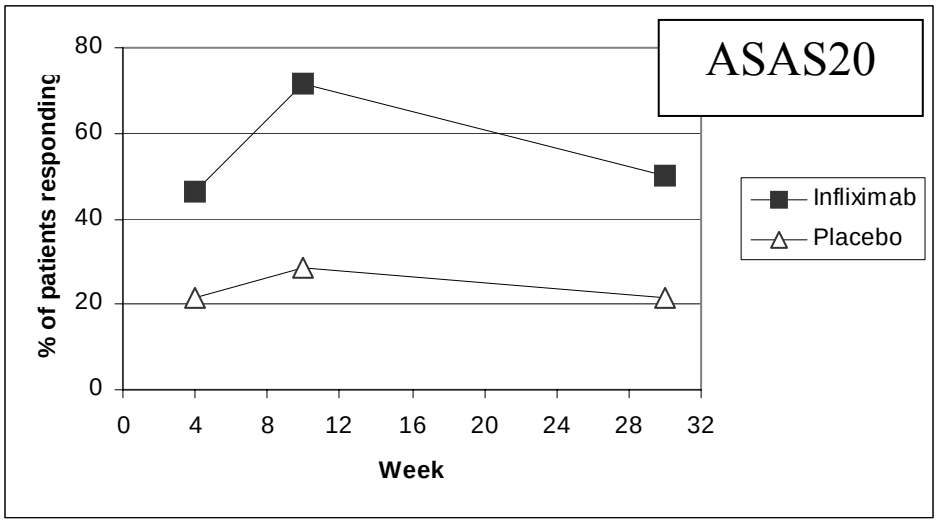


Figure 3. Proportions of patients responding to therapy as measured by ASAS responses: ASAS20 and ASASBIO.



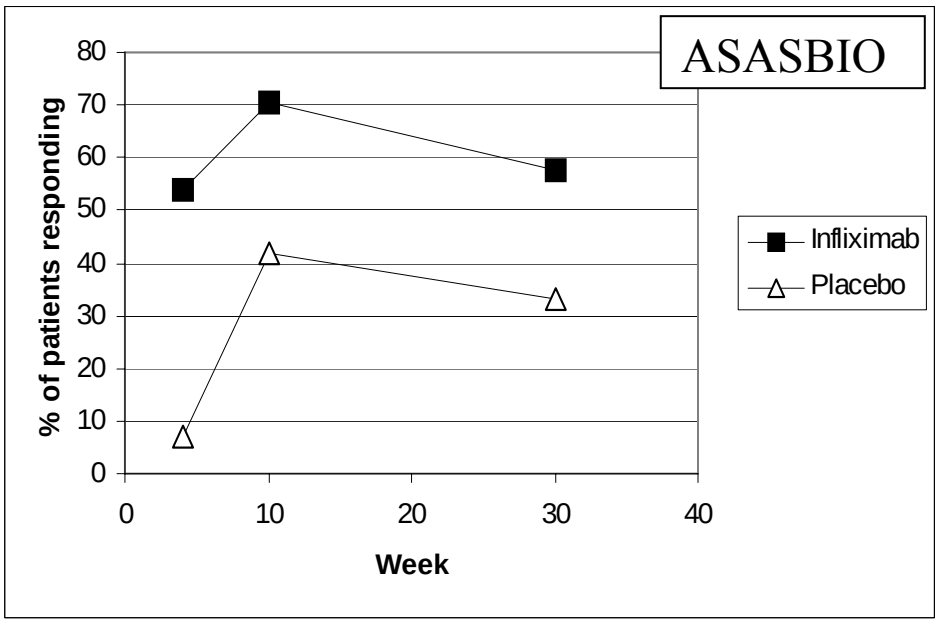



Figure 4. Association between change in BASDAI score and numbers of lesions resolved.

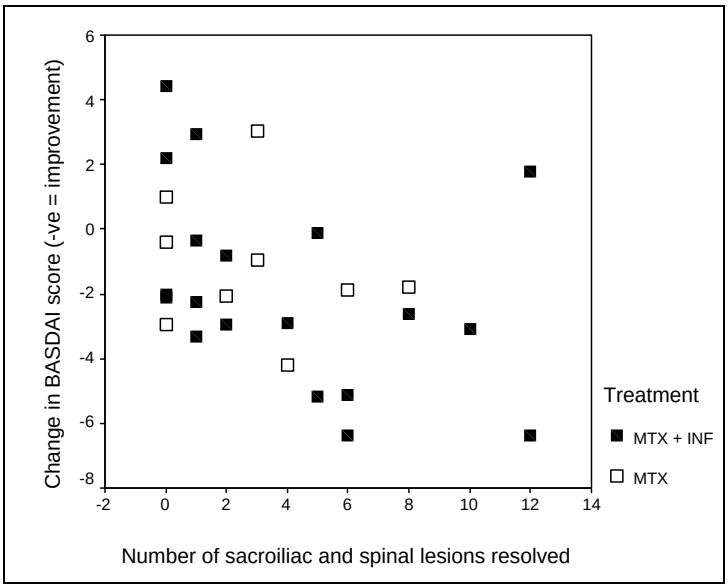
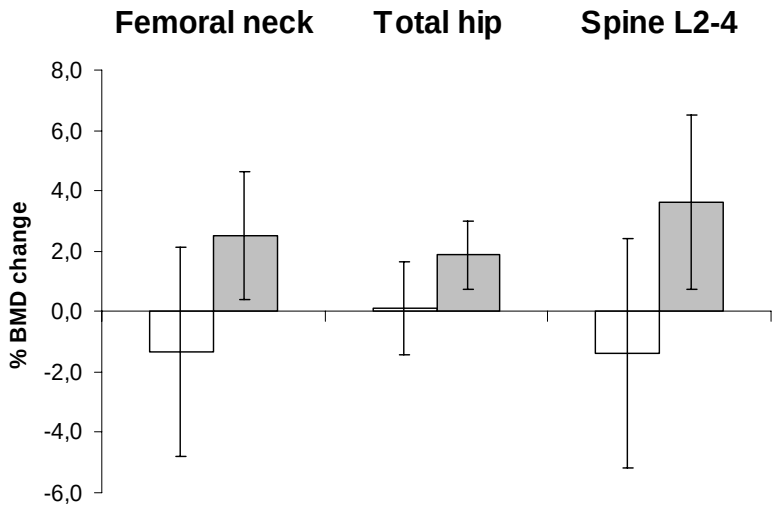


Figure 5. % change in BMD in both groups at week 30.




| | | Femoral neck | Total hip | Spine L2-4 |
|---|---|---|---|---|
| □ Placebo | BMD (SD) | 0.91 (0.12) | 0.92 (0.12) | 1.16 (0.13) |
| | Δ%BMD (SEM) | -1.34 (1.76) | 0.11 (0.79) | -1.38 (1.93) |
| ▨ Infliximab | BMD (SD) | 0.89 (0.15) | 0.89 (0.17) | 1.15 (0.29) |
| | Δ%BMD (SEM) | 2.52 (1.08) | 1.88 (0.58) | 3.60 (1.47) |

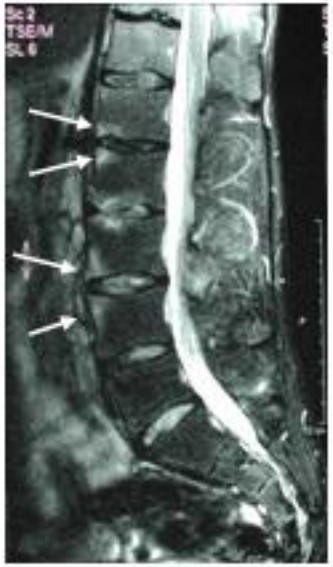



Fig 4A

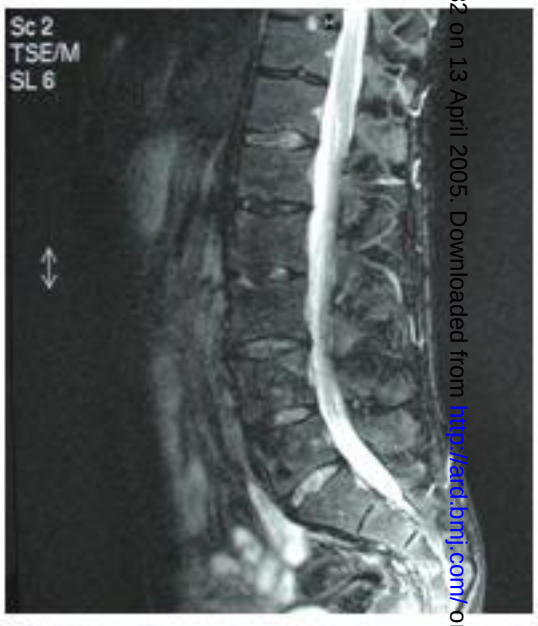



Fig 4B

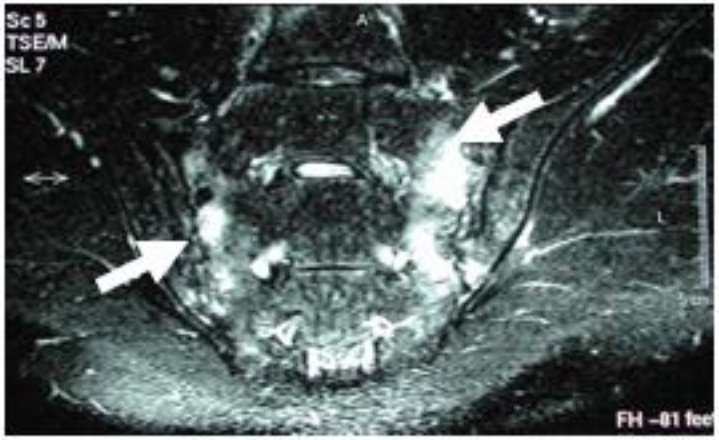



Fig 4C

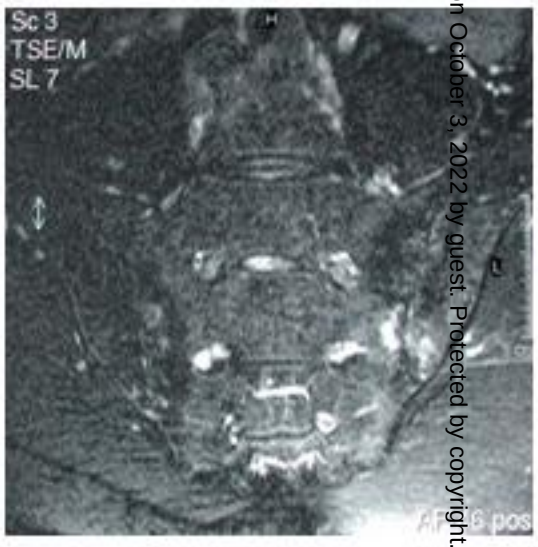



Fig 4D

Fig 6A. T2-weighted fat-suppressed (FS) sagittal sequence of the lumbar spine of a patient showing acute Romanus lesions (thin white arrows) at the anterior inferior aspects of L1 and L3, and anterior superior aspects of L2 and L4 vertebral bodies. Fig 6B. Illustrates complete resolution of the lesions after treatment with infliximab and methotrexate. Fig 6C. T2-weighted FS coronal oblique image of the sacroiliac joint of another patient showing active sacroiliitis (thick white arrow) which has improved markedly after treatment with infliximab and methotrexate (Fig 6D).