

Ultrasonographic measures of synovitis in an early phase clinical trial: a double-blind, randomised, placebo and comparator controlled phase IIa clinical trial of GW274150 (a selective inducible nitric oxide synthase inhibitor) in rheumatoid arthritis

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Abstract

Objectives

To test the sensitivity to change of ultrasonographic endpoints in early phase clinical trials in subjects with active rheumatoid arthritis (RA).

Methods

A double-blind, placebo and comparator controlled, randomised, two-centre study investigated the effect on synovial thickness and vascularity of 28 days repeat daily oral dosing of 60 mg of the inducible nitric oxide synthase inhibitor GW274150 or 7.5 mg prednisolone in RA. Fifty patients with DAS28 scores ≥ 4.0 were assigned to 3 treatment arms of 17, 19 and 14 (on placebo, GW274150 and prednisolone respectively). Synovial thickness and vascularity of all 10 metacarpophalangeal joints were assessed by ultrasonography using a semi-quantitative scale at baseline (Day 1), Day 15 and Day 28. Vascularity was also measured quantitatively by power Doppler area.

Results

At Day 28, the GW274150 group showed a trend towards reduction in synovial thickness compared with placebo, with an adjusted mean decrease of 33% ($p=0.072$); the prednisolone group decreased significantly by 44% ($p=0.011$). Similarly, there was a trend to reduced synovial vascularity with GW274150 by 42% compared with placebo ($p=0.075$); prednisolone resulted in a statistically significant decrease of 55% ($p=0.012$). There was a 55% decrease in power Doppler area for GW274150, compared with placebo although the result was not statistically significant ($p=0.375$). Prednisolone 7.5 mg resulted in a highly statistically significant decrease of 95% ($p=0.003$).

Conclusion

This study advocates the use of ultrasonographic measures of metacarpophalangeal joint synovitis as an endpoint for clinical studies assessing therapeutic potential of new compounds in small patient cohorts over 28 days.

Key words

rheumatoid arthritis, ultrasonography, corticosteroids, power Doppler, metacarpophalangeal joint

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Introduction

Developing new therapeutics for rheumatoid arthritis (RA) involves a programme of clinical trials designed to assess safety and efficacy. Traditionally, clinical benefit is initially assessed in terms of improvement in the symptoms and signs of disease by means of regulatory endpoints that include composite measures of disease activity, such as the Disease Activity Score (DAS28) (1), a continuous measure, and ACR (American College of Rheumatology) categorical responses (2). The components of these composite scores evaluate various aspects of local and systemic inflammation such as the number of tender and swollen joints, a measure of acute phase response, and the patients' own global assessment of their condition. However, many of the component measurements are subjective, as well as relatively imprecise and insensitive to change. In general, their use necessitates lengthy clinical trials using large cohorts of patients to evaluate new therapeutic compounds. A consequence of this approach is that drug development programmes become very costly and time consuming. Of further general concern in drug trials, even compounds that have progressed as far as phase III have an unacceptable failure rate that is reported to be as high as 60% in the case of lung cancer studies (3). Therefore, the development of novel, more reliable means of evaluating response to therapy in early phase trials that inform a decision to abandon or continue further testing would have the potential advantage of enriching for success in later clinical trials employing regulatory endpoints necessary for drug licensing.

High frequency ultrasound (HFUS) with power Doppler Ultrasound (PDUS) is a sensitive imaging method for objectively determining both synovial thickening and increased vascularity in RA (4-6). In the present study, we investigated the value of this technology to estimate treatment effects in the early development of novel therapeutics for RA. The drug tested was GW274150, a potent, highly selective inhibitor of inducible nitric oxide synthase (iNOS). Elevated levels of nitric

oxide (NO) and several mediators of NO metabolism have been found in serum, tissues and synovial fluid of RA subjects which suggests that NO may be contributing to the inflammatory processes, tissue damage, and pathology of RA (7-12). GW274150 has shown a beneficial effect in the collagen-induced arthritis (CIA) mouse model. GW274150 showed a decrease in paw swelling, an improvement in the histological score and reduction in radiographic damage compared to CIA mice treated only with vehicle (13). In our study, GW274150 was evaluated over a short period of time and with a view to achieving a clear interpretation of findings. GW274150 was compared in parallel to both placebo and prednisolone as an active comparator as the latter is an effective, quick-acting treatment of rheumatoid arthritis. The aims of this early phase clinical trial were to determine the efficacy of GW274150 in subjects with RA in comparison with low dose prednisolone and to test the sensitivity to change of ultrasonographic endpoints in this context.

Patients and methods

The study was a double-blind, randomised, placebo and comparator controlled, two-centre study to investigate the effect on synovial thickness and vascularity of 28 days repeat daily oral dosing of 60 mg of GW274150 or 7.5 mg prednisolone in RA subjects (GSK protocol number RA4104917 and Clinical Trials number NCT00379990). The dose of GW274150 60 mg daily was chosen as an earlier pharmacokinetic study demonstrated that RA subjects dosed with a single oral 90 mg dose resulted in higher systemic exposure than observed in healthy and asthmatic subjects (Clinical Trials number NCT00370435). All subjects had a diagnosis of RA according to the revised 1987 ACR criteria (14), disease activity defined as DAS28 (ESR) ≥ 4.0 , and at least one metacarpophalangeal joint (MCPJ) with either ultrasound detectable vascularity or synovial thickening or both at the screening visit. Ideally the cut-off of DAS28 ≥ 5.1 would have been preferred, however, due to the effective use of DMARD and

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biological therapies in Europe it is becoming increasingly difficult to recruit patients with severe disease activity. Therefore DAS28 ≥ 4 was chosen as a compromise between successful recruitment and selecting subjects with levels of disease activity amenable to demonstrate a treatment response to therapeutic intervention. Despite the requirement of DAS28 ≥ 4 at screening, in each treatment arm the mean DAS28 score at baseline was ≥ 5.1 . If subjects were taking small molecule disease-modifying anti-rheumatic drugs (DMARDs) then they had to be on stable doses for 8 weeks prior to enrolment and could continue taking these DMARDs during the study. Subjects who had received oral glucocorticoids within 8 weeks, intramuscular glucocorticoids within 6 weeks, infliximab or adalimumab within 3 months and etanercept or anakinra within 1 month of enrolment were excluded from the study. Non-steroidal anti-inflammatory drugs (NSAIDs) at stable doses for 2 weeks prior to the screening visit and throughout the study duration were permitted. Acetaminophen (paracetamol) was permitted for breakthrough pain, but NSAIDs were not to be taken on an as-needed basis. Subjects were assigned to study treatment in accordance with the randomisation schedule generated using the Randall system. The safety of subjects was assessed by clinical and laboratory examination and adverse event (AE) reporting during the study period.

All subjects gave informed written consent to participate in the study. The study design was performed according to the Helsinki Declaration and approved by the institutional review board for human research.

Ultrasonographic imaging

Using the Esaote Technos Plus MPX ultrasound machine with a 13MHz linear array probe (LA424 14-8) at both sites, subjects underwent ultrasonographic assessment of all 10 MCPJs in the transverse plane over the dorsal surface at Day 1 (baseline), Day 15 and Day 28. To obtain the transverse plane image, the probe is first aligned in the longitudinal axis to place the maximum

height of the dorsal triangular structure (an inverted triangular area whose margins are the metacarpal head, the phalangeal base and the joint capsule superiorly) (15, 16) in the centre of the screen image. At this point the probe is rotated by 90° to achieve the transverse view. Care was taken when scanning to avoid undue pressure with the probe in case this altered blood flow in the joint. This was achieved by maintaining a distance of at least 1 mm of gel between the probe and the subject as visualised on the US monitor.

The time of day of the measurements at each visit was within 1 hour of the time of the baseline visit. The same sonographer (MS) scanned the joints of each subject at all visits at the Kennedy Institute, London, to ensure consistency. At the Institute of Rheumatology, Belgrade, the same sonographer (SP) scanned the joints of each subject at all visits. Both sonographers were blinded to the randomisation of the patients to their respective groups and to the clinical examination for DAS28 scoring.

HFUS still images were recorded for synovial thickness in the transverse plane for future analysis. US images were anonymised and evaluated in the UK by MS. HFUS images of synovial thickness in the transverse plane were assigned a score of 0–5 by MS. A semiquantitative 0–5 scale of transverse synovial thickness was utilised and related to hypoechoic areas, as follows: grade 0, normal; grade 1, minimal; grade 2, mild; grade 3, moderate; grade 4, marked; grade 5 maximal (Fig. 1A). Images were graded against this library of representative images, *i.e.* for each acquired image MS visually estimated the magnitude of the synovial thickness area, compared this with the library, decided which representative image was the closest fit and allocated a score. The total synovial thickness score was calculated as the sum of individual joint scores (minimum score of 0 and a maximum of 50).

All MCPJs were scanned in the power Doppler mode (13 MHz) with a fixed PRF of 750 Hz, Gain of 133 and Medium Wall Filter. A three-second digital film clip was recorded for each MCP joint because the power Doppler signal

fluctuates with the cardiac cycle and the aim was to evaluate the maximum signal. Anonymised images demonstrating maximal synovial vascularity were assessed on a semi-quantitative scale relating to colour signal (grade 0, normal; grade 1, minimal; grade 2, mild; grade 3, moderate; grade 4, marked; grade 5 maximal) (Fig. 1B). For each selected image MS visually estimated the amount of coloured pixels within the joint capsule, compared this with the library, decided which representative image was the closest fit and allocated a score. The total vascularity score was calculated as the sum of individual joint scores (with a minimum score of 0 and a maximum of 50). In addition, the images were analysed using in house software written in MATLAB (The Mathworks, Waltham, MA) allowing a rapid automated measurement of number of colour pixels in a region of interest (ROI). The ROI on each transverse power Doppler MCP image enveloped the MCP joint synovium from the upper margin of the joint capsule to the lowest point of the triangular structure, excluding digital vessels and reflection artefacts (Fig. 2). This gave a quantitative value referred to as the power Doppler Area (PDA) which was summated for all 10 MCP joints to give the total PDA. Four-point semiquantitative scales (0–3) have been published in the literature for both synovial hypertrophy and power Doppler (17–19). However, the aim of the 6 point scale was to explore the potential of improving the sensitivity of these ultrasonographic qualitative outcome measures. The scale has been used in a recent study in which anonymised images were read by three investigators (a rheumatology research fellow, a consultant radiologist and a consultant rheumatologist) who together assigned a score of 0–5 by consensus. This study demonstrated that PDA correlated with baseline DAS28 scores ($r=0.42$, $p<0.05$) and that this measure was more sensitive at detecting change than other biomarkers (ESR, CRP, and VEGF) in patients with active RA treated with low dose corticosteroid over a two-week period (20). Baseline synovial vascularity and synovial thickness indices correlated more closely with

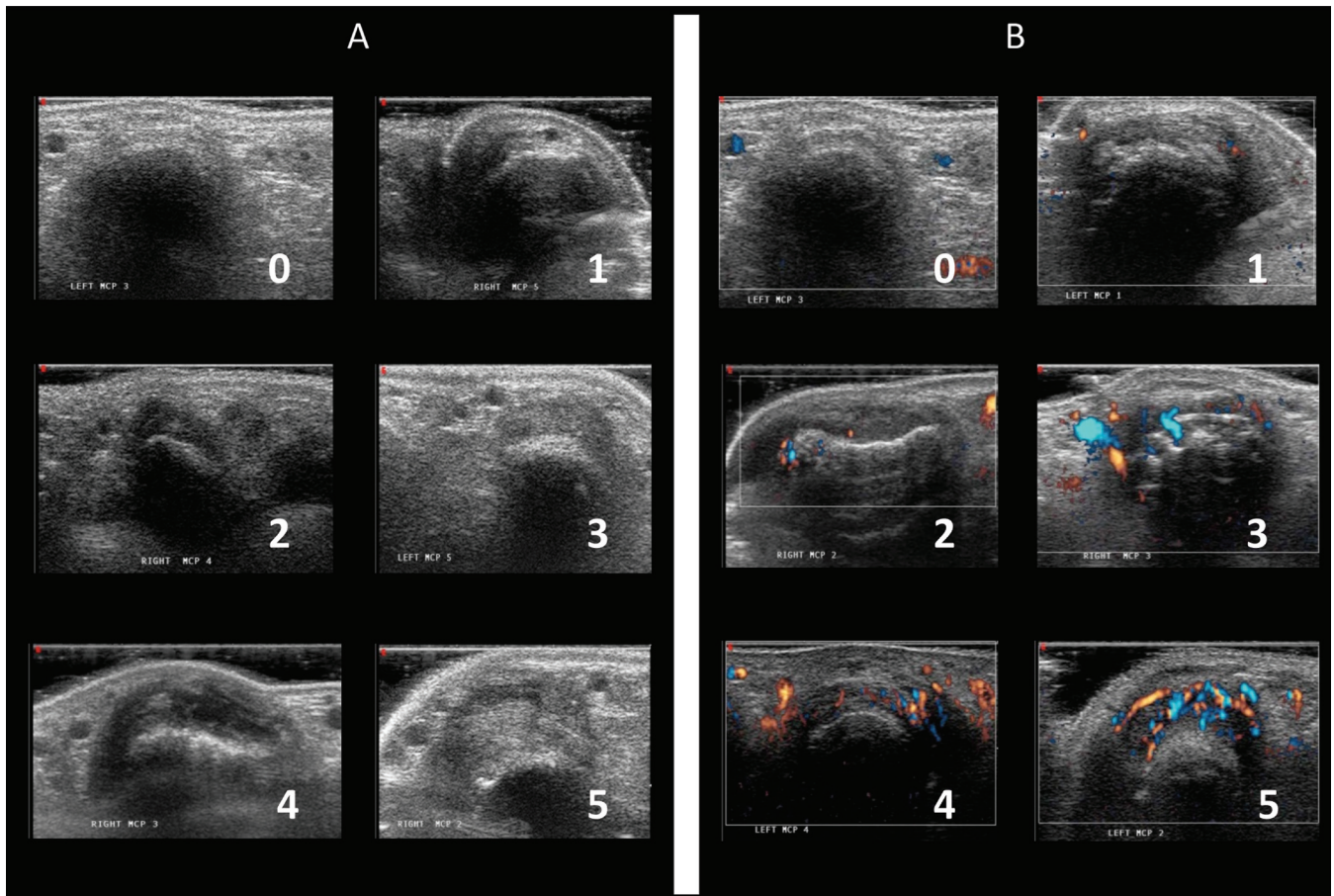


Fig. 1 (A) Semiquantitative ultrasound scale of synovial thickness (0–5) relating to hypoechoic areas: grade 0, normal; grade 1, minimal; grade 2, mild; grade 3, moderate; grade 4, marked; grade 5 maximal. (B) Semiquantitative ultrasound scale of vascularity (0–5) relating to power Doppler signal: grade 0, normal; grade 1, minimal; grade 2, mild; grade 3, moderate; grade 4, marked; grade 5 maximal. Images were cropped to focus on the area of interest. Reproduced with permission from the Journal of Rheumatology (Larché and Seymour *et al.* (20): *J Rheumatol* 2010; 37(12): 2493–501).

baseline DAS28 scores ($r=0.51$, $p<0.01$ and $r=0.54$, $p<0.001$, respectively); the former had similar sensitivity to CRP. The ultrasonographic outcome measures in the transverse view were chosen as these have previously shown their utility in differentiating two groups in a randomised placebo controlled trial (21).

Laboratory assessments and evaluation of clinical response

Blood samples were collected at Day 1, 15 and 28 for determination of the erythrocyte sedimentation rate (ESR) and serum C-reactive protein (CRP) by the Westergren method and by a laboratory Latex test respectively. DAS28(ESR) scores were measured at all three time points by an independent assessor. Blood samples were collected to determine plasma concentrations of GW274150. Besides sampling on Day 15 and Day 28, an additional trough

sample was obtained in a blinded manner from each subject on Day 8.

Statistical analysis

The sample size was chosen such that 15 subjects per treatment arm would detect a decrease of 60% in total PDA for GW274150 *versus* placebo, with 90% power at the two-sided 5% level. This calculation was based on a between subject standard deviation of 0.74 seen in a previous study (21). Formal statistical analyses were carried out on the \log_e -transformed imaging endpoints, *i.e.* total thickness score, total vascularity score, and PDA using a repeated measures model including country as a covariate. Plots of adjusted geometric means with 95% confidence intervals (CI) and adjusted treatment ratios with 95% CI were produced. Due to the necessity of \log_e -transforming the data and to the presence of some 0 values within the data for total synovial vas-

cularity score, total synovial thickness score and PDA, a sensitivity analysis was carried out by reanalysing the data imputing all 0 values to 1. The above statistical analyses were performed using SAS for UNIX, version 8.2 (SAS Institute, Cary, North Carolina, USA). Within scan intra-reader reproducibility was determined by randomly selecting 18 anonymised US data sets of 10MCPJs (9 UK and 9 Serbia). MS reread the images and clips two years after his first reading in a blinded fashion, *i.e.* for PD clips MS once again determined the frame that demonstrated maximum vascularity which was subsequently scored semi-quantitatively and quantitatively. Joint by joint comparisons and composite score (summation of 10MCPJs) comparisons were made between the second reading and the first reading for each US endpoint. Intraclass correlation coefficient (ICC) values were determined using the free

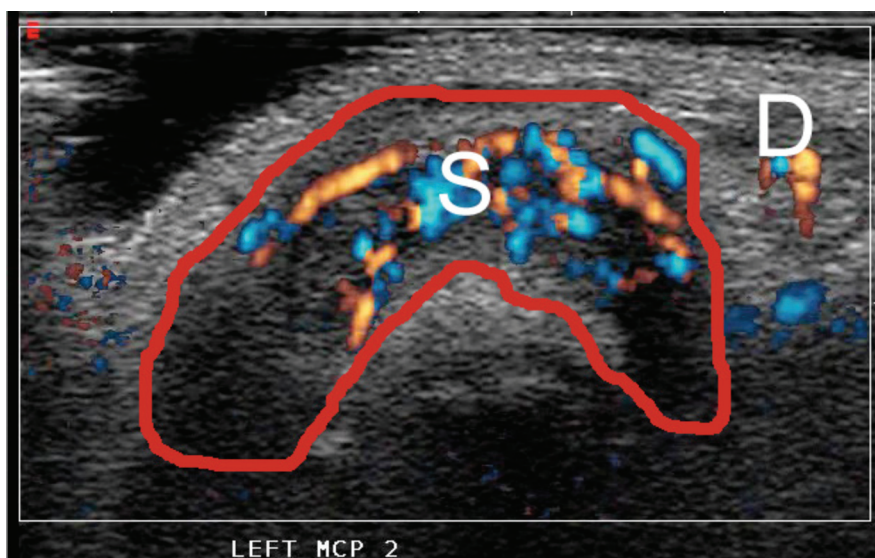


Fig. 2. A MCP joint scanned in the transverse plane demonstrating grade 5; maximal synovial vascularity. The region of interest (ROI) is within the red line. D and S denote a digital vessel and synovial vascularity, respectively.

Table I. Subject characteristics.

	Placebo	60 mg GW274150	7.5 mg prednisolone
Number of subjects planned, n.	15	15	15
Number of subjects randomised, n.	17	19	14
Number of subjects completed, n (%)	14 (82)	15 (79)	14 (100)
Age in years, mean (range)	57.1 (34–74)	56.4 (38–79)	53.8 (25–68)
Female, n. (%)	15 (88)	15 (79)	10 (71)
Rheumatoid factor positive, n. (%)	12 (71)	15 (79)	8 (57)
Anti-CCP antibodies positive, n. (%)	13 (76)	18 (95)	8 (57)
% on methotrexate, n. (%)	15 (88)	17 (89)	11 (79)
% on > 1 DMARD n. (%)	4 (24)	5 (26)	2 (14)
Baseline DAS28 (ESR), mean (range)	6.04 (4.2–7.1)	6.27 (3.8–7.9)	5.64 (3.7–7.0)
Baseline ESR (mm/hr), mean (range)	35.1 (8–62)	26.7 (8–76)	41.8 (14–96)

Table II. Summary of the statistical analysis of DAS28 scores.

Time point	Adjusted means			Differences of adjusted means (95% CI)	
	Placebo (n=17)	GW274150 60 mg (n=19)	Prednisolone 7.5 mg (n=14)	GW274150 60 mg placebo	Prednisolone 7.5 mg placebo
Day 15	5.75	5.49	4.97	-0.26 (-0.82, 0.31) <i>p</i> =0.367	-0.77 (-1.35, -0.19) <i>p</i> =0.010
Day 28	5.39	4.96	4.41	-0.42 (-1.22, 0.37) <i>p</i> =0.290	-0.98 (-1.78, -0.18) <i>p</i> =0.018

online calculation software provided by the Department of Obstetrics and Gynaecology of the Chinese University of Hong Kong (22).

Results

Clinical evaluation

Fifty subjects, split into 3-treatment arms of 17, 19 and 14 subjects (on

placebo, GW274150 60 mg and prednisolone 7.5 mg respectively) were recruited into the study. Clinical and demographic characteristics of the study subjects are shown in Table I. Forty-three subjects (86%) completed the study. Seven subjects were withdrawn from the study: 5 subjects due to AEs and the remaining 2 subjects due

to the other reasons. Out of the 5 subjects who withdrew due to an AE, one was on placebo and withdrew because of severe abdominal pain considered to be related to the study drug by the investigator prior to unblinding. The other 4 subjects who withdrew due to AEs were on GW274150 and the reasons for withdrawal were a moderate vomiting event (considered to be related to the study drug), a moderate allergic rash (not considered to be related to the study drug), a moderate urticaria (considered to be related to the study drug) and mild increased alanine aminotransferase increase (not considered to be related to the study drug). Headache was the most frequently reported AE (5 subjects following Placebo and 2 subjects following 60 mg GW274150). GW274150, dosed at 60 mg once daily for 28 days, was generally safe and well tolerated. There were no deaths, no serious adverse events or pregnancies reported in this study. Steady state pharmacokinetics of GW274150 was achieved by Day 8. Inter-subject variability in steady state drug levels was low/moderate. Exposure in this patient population was similar to that observed in healthy subjects and asthmatics dosed with 90 mg GW274150.

At day 28 there was a 0.42 decrease in the DAS28 score in the GW274150 group compared with placebo, however this was not statistically significant (*p*=0.290) (Table II). For subjects taking prednisolone, the DAS28 showed a statistically significant adjusted mean decrease of 0.77 (*p*=0.010) at Day 15 and 0.98 (*p*=0.018) and at Day 28 compared with placebo (Table II).

Ultrasound

At Day 28 GW274150 showed a trend towards reduction in synovial thickness compared with placebo, with an adjusted mean decrease of 33% (*p*=0.072), *i.e.* an adjusted treatment ratio of 0.67. Prednisolone 7.5 mg showed a statistically significant decrease of 44% (*p*=0.011) (Fig. 3A). GW274150 decreased synovial vascularity by 42% compared with placebo but the reduction was not statistically significant (*p*=0.075). However, prednisolone 7.5 mg resulted in a statistically significant

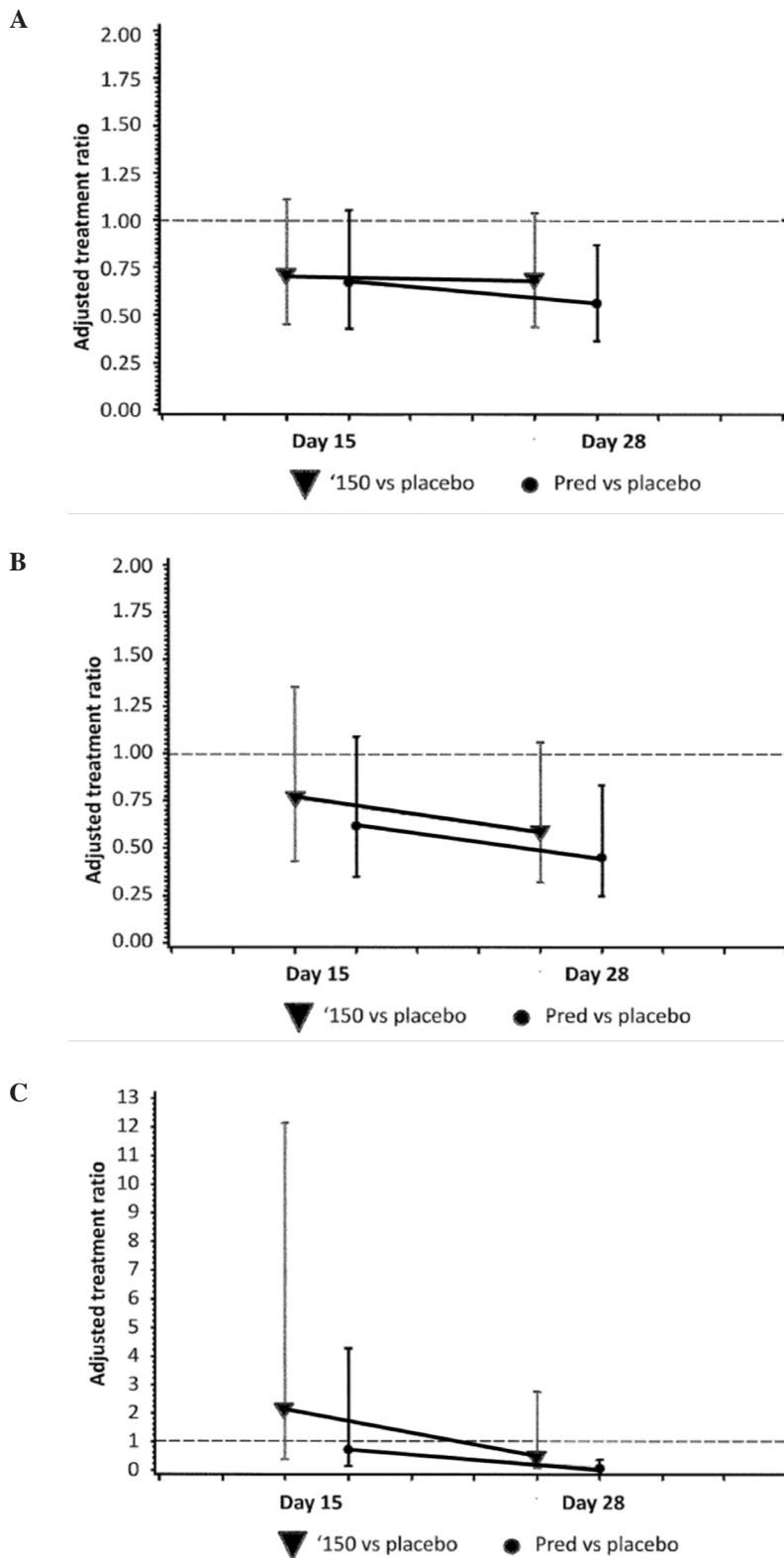


Fig. 3. A. Plot of adjusted treatment ratios of total synovial thickness score data with 95% confidence intervals by day and treatment comparison (with imputed values for zero [1]). B. Plot of adjusted treatment ratios of total vascularity score data with 95% confidence intervals by day and treatment comparison (with imputed values for zero [1]). C. Plot of adjusted treatment ratios of total power Doppler area data with 95% confidence intervals by day and treatment comparison (with imputed values for zero [1]). Values on the y axis below 1 indicate reductions in the respective ultrasonographic measures at Days 15 and 28.

decrease of 55% ($p=0.012$) (Fig. 3B). There was a 55% decrease in PDA for GW274150, compared with placebo although the result was not statistically significant ($p=0.375$). Prednisolone 7.5 mg resulted in a highly statistically significant decrease of 95% ($p=0.003$) in PDA (Fig. 3C).

Within scan intra-reader reliability: For joint by joint analysis, the ICC for 180 paired readings was excellent for all US endpoints; PDA 0.99, PD vascularity 0.95, and HFUS synovial thickness 0.83. For 10MCPJ composite scores, the ICC for 18 paired readings was also excellent for all US endpoints; PDA 0.98, PD vascularity 0.85, and HFUS synovial thickness 0.85.

Discussion

For the purposes of early stage testing of novel therapeutics, we require a sensitive and reliable method to distinguish between treatment groups in cohort studies that permit small numbers of subjects and are reliable indicators of efficacy at an early time point. Ideally, such measures would also be predictive of a longer-term response to repeated medication and give an early indication of disease modification. MRI is considered the new gold standard for the detection of joint inflammation. MRI rates of early synovial enhancement (RESE) after injection of gadolinium have been shown to correlate closely with the histological grade of synovitis (23, 24). In turn, the synovial vascular signal on PDUS is closely correlated with the RESE, calculated from dynamic contrast enhanced MRI on the same day, in RA MCPJs (25, 26). These observations suggested that there might be potential value in visualising MCPJs of RA subjects using ultrasonography as a quick, non-invasive and relatively inexpensive alternative to contrast enhanced MRI.

In recent clinical studies we and others have explored the potential of HFUS with PDUS to measure synovial thickening and vascularity and thereby deliver a reliable synovitis signal early during the course of therapy which correlates with clinical outcome (21, 27-29). Encouraged by these findings using therapies of proven benefit in RA,

we undertook the present study to investigate the sensitivity of ultrasonographic endpoints to change in the context of an early phase clinical trial in subjects with active rheumatoid arthritis.

Treatment with GW274150 demonstrated a reduction in all 3 ultrasonographic measures and DAS28 score but the results were not statistically significant. The reason for this could have been threefold. Firstly, the dose of GW274150 was insufficient to produce clinically relevant levels of intraarticular iNOS inhibition. Secondly, in RA NO may be a marker rather than a mediator of inflammation akin to the possible situation in asthma. Exhaled breath nitric oxide is increased in asthma (30, 31, 32) and is produced predominantly by iNOS (33-35). The effect of GW274150 on asthma was assessed in a double-blind, randomised, double-dummy, placebo controlled study. Although GW274150 significantly reduced predose exhaled breath NO after 14 days compared with placebo, it did not affect airway hyperreactivity or airway inflammatory cell numbers after allergen challenge in subjects with asthma (36). Lastly, the time period of the study was too short and/or there were insufficient subject numbers to demonstrate a significant GW274150 effect. Parallel scan inter-reader reliability (involves: 1 patient, 2 ultrasonographer-readers each acquire a scan set independently, same 2 ultrasonographer-readers each read their own acquired scan set independently) is the most rigorous test of reproducibility as it examines both the acquisition and reading processes. The result of this assessment would be especially relevant to multi-site clinical trials using the same model of US machine and settings with different but harmonised ultrasonographer-readers. A limitation of the present study was that this measure of reliability was not written into the protocol but we aim to do this in future studies. It would have been worthwhile to have incorporated US assessments of MCPJs in the longitudinal plane as utilising only the transverse may have over or underestimated the power Doppler signal and synovial thickness. In future studies we plan to include both

planes and compare the ability of assessments in each plane to register a therapeutic response to treatment intervention in RA.

The current study advocates the use of ultrasonographic measures of metacarpophalangeal joint synovitis as a primary endpoint in early phase studies assessing the therapeutic potential of new compounds in small patient cohorts over a 28-day test period. In this study PDA was a more sensitive method for detecting change than other biomarkers indicative of its high potential as an early marker of therapeutic response. This study also illustrates the potential utility of US to stratify patient selection by detecting those with unequivocal baseline joint pathology with a potentially reversible inflammatory basis.

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