Should Tetracycline Treatment Be Used More Extensively for Rheumatoid Arthritis? Metaanalysis Demonstrates Clinical Benefit with Reduction in Disease Activity

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ABSTRACT. ***Objective.*** To compare the effectiveness of tetracycline antibiotics versus control (placebo or conventional treatment) in rheumatoid arthritis (RA) for the reduction of disease activity as defined by American College of Rheumatology criteria.

Methods. We searched Medline (1966–February 2002), Embase (1980–February 2002), and the Cochrane Controlled Trials Register (Issue 1, 2002 Cochrane Library). Reference lists of published trials were searched by hand for further identification of published reports and presentations at scientific meetings. Randomized controlled trials comparing tetracyclines to control (placebo or conventional disease modifying antirheumatic therapy) were selected for inclusion if at least one of the following outcomes was reported: tender joint count (TJC), swollen joint count, patient pain score by visual analog scale, patient global assessment of disease activity, physician global assessment of disease activity, eosinophil sedimentation rate (ESR) and C-reactive protein (CRP), joint space narrowing and erosions, adverse events, and quality of life as measured by the Health Assessment Questionnaire. Subjects were required to have RA as defined by the 1987 ARA criteria.

Results. Ten randomized controlled trials including 535 individuals were reviewed. Only 3 trials were considered high quality; elements of bias could not be excluded in the remainder. Tetracyclines, when administered for ≥ 3 months, were associated with a significant reduction in disease activity in RA as follows: for TJC, standardized mean difference (SMD) = –0.39, 95% CI –0.74, –0.05; and for acute phase reactants, ESR, SMD = –8.96, 95% CI –14.51, –3.42. The treatment effect was more marked in the subgroup of patients with disease duration < 1 year who were seropositive. There was no absolute increased risk of adverse events associated with tetracyclines: absolute risk difference = 0.10, 95% confidence interval (CI) –0.01, 0.21. No beneficial effect was seen on radiological progression of disease: for erosions, SMD = 0.17, 95% CI –0.29, 0.64. In addition, subgroup analysis excluding trials with doxycycline showed that minocycline alone had a greater effect on reduction of disease activity: for TJC, SMD = –0.69, 95% CI –0.89, –0.49; and for ESR, SMD = –10.14, 95% CI –14.72, –5.57.

Conclusion. Tetracyclines, in particular minocycline, were associated with a clinically significant improvement in disease activity in RA with no absolute increased risk of side effects. Unfortunately, the information available was inadequate to allow a detailed analysis of individual side effects in the studies. Further research is warranted to compare these agents to newer disease modifying drugs for comparable safety, efficacy, and cost-effectiveness. (J Rheumatol 2003;30:2112–22)

Key Indexing Terms:
MINOCYCLINE DOXYCYCLINE RHEUMATOID ARTHRITIS
RANDOMIZED CONTROLLED TRIALS METAANALYSIS

Rheumatoid arthritis (RA) is a chronic debilitating disease affecting up to 1% of the population. The pathogenesis is complex and incompletely understood. Chronic inflammation leads to joint damage with progressive deformity and disability in patients. Early intervention with disease modifying antirheumatic drugs (DMARD) is beneficial in mini-

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mizing longterm disability. DMARD are thought to have an immune-modulating effect reducing disease activity and progression. Commonly used DMARD include methotrexate (MTX), sulfasalazine (SSZ), gold, and antimalarials. The benefit in terms of reduction in disease activity and damage for RA has been established with respect to MTX[1], gold[2], SSZ[3], and hydroxychloroquine (HCQ)[4].

The new biologic agents infliximab[1,5] and etanercept[6] have led to impressive reductions in disease activity score and amelioration of structural damage. However, recent reports have highlighted the potential for adverse events with these agents, particularly the risk of infection and the reactivation of latent tuberculosis[7]. In addition, there are other issues concerning the use of these drugs, including the high cost.

Over a century ago, Osler proposed an infectious etiology for RA. It was purported that RA was caused by tuberculosis, which lead to the trial of gold as a DMARD for RA. SSZ was proven to be an effective DMARD for RA[3] and it had both antiinflammatory and antimicrobial properties. The salicylate component was thought to target joint inflammation and the sulfonamide to eradicate any underlying infection. Indeed, several antibiotics have been used as treatment for RA, including dapsone[8], ceftriaxone[9], metronidazole[10], and rifampicin[11]. Tetracycline was first used to treat RA in 1971[12]. The original rationale for this was eradication of mycoplasma infection, which was implicated at that time in the disease pathogenesis. In the early 1990s, several open label trials were conducted suggesting a possible therapeutic benefit for minocycline in the treatment of RA[13,14].

Tetracyclines, in particular minocycline and doxycycline, have antiinflammatory activities independent of their antimicrobial properties. This has complicated the interpretation of response in RA trials. Recently, Amin, *et al*[15] demonstrated that minocycline and doxycycline downregulate type 2 nitric oxide synthase, an important mediator in collagen degradation. Minocycline has also been shown to upregulate interleukin 10, a potent inhibitory cytokine in synovial tissue[16]. Both minocycline and doxycycline have direct suppressive effects on B and T cell function[17,18] and inhibit matrix metalloproteinase (MMP) 1 and 11[19,20], thus potentially limiting cartilage degradation and collagenase activity. A few randomized controlled trials (RCT) have been performed in recent years as a consequence of these observations, and the use of tetracyclines for treatment of RA has gained popularity. Although there have been some adverse events reported with tetracycline therapy, it is possible that they may still represent a safer and more cost-effective alternative to the current treatment approaches for RA. Therefore, we performed a metaanalysis to examine the evidence for their use in RA to ameliorate disease activity.

The primary objective of this metaanalysis was to compare the effectiveness of tetracycline therapy versus control (placebo or conventional treatment) for active RA in the prevention or reduction of disease activity as defined by the 1995 American College of Rheumatology (ACR) response criteria[21]. Secondary objectives were to compare the effectiveness of tetracyclines versus control on (1) radiological disease progression; (2) incidence of adverse events; and (3) change in serological markers, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP).

MATERIALS AND METHODS

Selection of data. The criteria for considering studies for this review were based on the following:

1. Types of studies: Only randomized clinical trials comparing tetracycline antibiotics orally or intravenously to placebo or conventional DMARD therapy in RA were considered for inclusion.
2. Types of participants: Subjects were at least 16 years of age and had to satisfy the 1987 modified American Rheumatism Association criteria for RA[22]. Patients were required to have active disease as shown in the following variables: presence of tender joints [tender joint count (TJC)] or swollen joints [swollen joint count (SJC)], morning stiffness, and increased acute phase reactants (ESR, CRP).
3. Types of interventions: Tetracycline antibiotics versus control (placebo or conventional treatment).
4. Types of outcome measures: Primary outcome measures were specified to include one or more of the following: ACR core set of disease activity measures for RA clinical trials that have been endorsed by the European League Against Rheumatism (EULAR) and the Outcome Measures in Rheumatology Clinical Trials (OMERACT)[21,23]. These include the following outcome measures: TJC, SJC, patient perception of pain level (PT PAIN), patient global assessment of disease activity (PT GA), physician global assessment of disease activity (MD GA), ESR, CRP, and radiographic change of bone and joint damage [erosions and joint space narrowing (JSN)].

Secondary outcome measures included adverse side effect profile and disability as measured by the Health Assessment Questionnaire (HAQ).

Search strategy. RCT using tetracycline antibiotic therapy for treatment of RA were identified from Medline (1966 to February 2002) using the following MeSH headings: antibiotics, tetracyclines, arthritis, rheumatoid, and randomized controlled trials. Other databases were searched including Embase (1980 to February 2002) and the Cochrane Controlled Trials Register (Issue 1, 2002 Cochrane Library). Reference lists of published trials were searched by hand for further identification of published work and presentations at scientific meetings. The search strategy was conducted as recommended by Haynes, *et al*[24]. No language restriction was applied. Trials were limited to humans and published reports only. No dosage administration regimen was specified. The candidate articles (n = 37) were screened to identify articles eligible for inclusion in the review.

All published articles identified as potentially relevant by the literature search were assessed by 2 reviewers (MS, CP-T) to select trials that fulfilled the inclusion criteria. Differences were resolved by consensus.

Validity assessment. Assessment of trial quality was qualitative and addressed the following issues: adequacy of concealment of random allocation; blinding of outcome assessor and patients for all outcomes reported; and blinding caregiver and patient for intervention and completeness of followup. To satisfy this last criterion, authors were required to have > 80% followup assessments on all subjects enrolled. It was insufficient for trials to merely use the double-blind or triple-blind terminology, they were required also to explicitly state who was blinded and how (this pertained to investigators, patients, and outcome assessors). A trial was considered high quality if it satisfied all 4 criteria, medium quality if it satisfied 3 out of 4 criteria, and poor quality if it satisfied 2 or fewer criteria.

Data abstraction. The following information was abstracted from each

selected trial using a predesigned data abstraction form: trial design, characteristics of study population, treatment regimen and duration, and baseline and end of study outcome measures.

Differences in data extraction were resolved by consensus with a third reviewer (RI).

Quantitative data synthesis. Data on the outcome measures from each trial were pooled where possible to obtain the overall estimate of the effectiveness of tetracycline antibiotics in RA therapy. Wherever possible, the analyses were based on intention-to-treat data from individual trials. For continuous data, results were presented as a weighted mean difference (WMD). Where different scales were employed (i.e., TJC, SJC) the standardized mean differences (SMD) were reported. For dichotomous data, relative risks (RR) were calculated. For adverse events the absolute rate was reported. Homogeneity of the data was calculated using the chi-square test at n – 1 degrees of freedom, with the significance level of $p < 0.1$. Metaanalysis was performed using Revman 4.1 software, and conducted according to a fixed effect model except where heterogeneity existed. In such cases, a random effects model was used.

Subgroup analysis. The following subgroup analyses were planned to test the robustness of the results: (1) Exclusion of trials where an active comparator was used as the control. (2) Exclusion of those trials where the tetracycline antibiotic used was doxycycline. (3) Exclusion of trials where the patient cohort had early onset disease and were exclusively seropositive.

RESULTS

Study characteristics. Details of the studies are provided in Table 1 and Figure 1 (trial flow diagram). The initial Medline search, using strategy as stated, identified 35 articles. Two further studies were identified from the reference lists of the articles retrieved. Thus, a total of 37 studies were assessed. Twenty of these were excluded on first pass by the reviewer as they were not RCT, and 5 RCT were subsequently excluded as tetracycline antibiotics were not used. Ten studies involving 535 patients with RA met the inclusion criteria.

Two of the studies (n = 106 subjects) described early onset, seropositive RA[25,26]. The studies were performed in several different countries — The Netherlands, USA, India, and Sweden. The tetracyline antibiotics used were minocycline[26,27] and doxycycline[28-30] and one trial used tetracycline[12]. Minocycline was used orally and varied in dose from 100 to 200 mg/day (Table 2). Doxycycline was given intravenously (IV) at a dose of 200 mg/day for days 1–21 and 200 mg IV once weekly from week 4 to 11 in one study[30]. Doxycycline was administered orally in 2 trials[28,29] and varied in dose from 200 mg/day for 26 weeks to 50 mg BID for 36 weeks. All trials except 2 compared antibiotic to placebo[26,28]. In these 2 exceptions, O'Dell, *et al* compared antibiotic to HCQ[26], and Sreekanth, *et al* compared antibiotic to MTX[28]. In the study by St. Clair, *et al*[30], in addition to a placebo arm a third arm compared doxycyline to azithromycin as the control antibiotic. In the study by van der Laan, *et al*[29], the eligible patients were distributed among 4 groups (randomization was not clearly described): group A, doxycycline 0–12 weeks, placebo 12–24 weeks, and doxycycline 24–36 weeks; B, doxycycline 0–24 weeks and placebo 24–36 weeks; C, doxycycline for 36 weeks; and D, placebo for 36 weeks. Thus, it was possible only to combine the results from the groups C and D (n = 34 patients) in the metaanalysis.

Apart from one trial[26], the duration of treatment varied from 11 to 52 weeks; in that exception the study duration was 2 years. Both studies looking at radiological outcomes treated subjects for 48 weeks. In the study by O'Dell, *et al*[26], radiological outcomes were measured, but were not mentioned in the results. One study treated patients for 52 weeks[12].

The assessment of individual studies detailing sample size, age, disease duration, seropositivity for rheumatoid factor, and disease severity is presented in Table 1. The mean disease duration ranged from 5.6 months to 12 years in the studies. The mean age of patients ranged from 33 to 58 years. Seropositivity ranged from 55% to 100% in the treatment groups and 70%–100% in the control groups. The presence of erosive disease at baseline varied from 65% to 97% in the control group and 70%–92% in the treatment

Table 1. Patient demographics and clinical characteristics.

Study	Subjects, n	Disease Duration, yrs		Age, yrs, mean		Rheumatoid Factor, %		Erosive Disease, %	
		Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
Skinner 1971	30	5*	9.5*	55†	52†	11/14	12/14	NG	NG
Kloppenburg 1994	80	12 ± 10	14	54 ± 12	58 ± 12	92	95	95	95
Tilley 1995	219	8.4 ± 8.6	8.8 ± 9.3	55 ± 12.8	53 ± 13.5	55	57	70	65
Kloppenburg 1996	65	11 ± 10	13 ± 9	54 ± 12	60 ± 12	97	87	92	97
O'Dell 1997	46	> 0.5–1	> 0.5–1	NG	NG	100	100	NG	NG
Bluhm 1997	219	8.4 ± 8.6	8.8 ± 9.3	55 ± 12.8	53.5 ± 13.5	55	57	70	65
Sreekanth 2000	35	NG		38.8 ± 7.7	33.3 ± 7.5	NG	NG	NG	NG
Van der Laan 2000	34	12 (4–47)	9 (1–47)	53 ± 11	57 ± 9	82	77	100	94
St. Clair 2001	31	> 0.5–12	> 0.5– < 12	NG	NG	NG	NG	NG	NG
O'Dell 2001	60	5.6 ± 3 mths	5.8 ± 3.8 mths	50.6 (20–71)	45.7 (23–72)	100	100	NG	NG

* Median disease duration; † median age. NG: not given. Trial Kloppenburg 1996 is an extension of Kloppenburg 1994 and trial Bluhm 1997 is an extension of trial Tilley 1995. However, all trials report on separate outcomes so are listed as individual trials here, but patients are only counted once towards the overall number of patients included in the review.

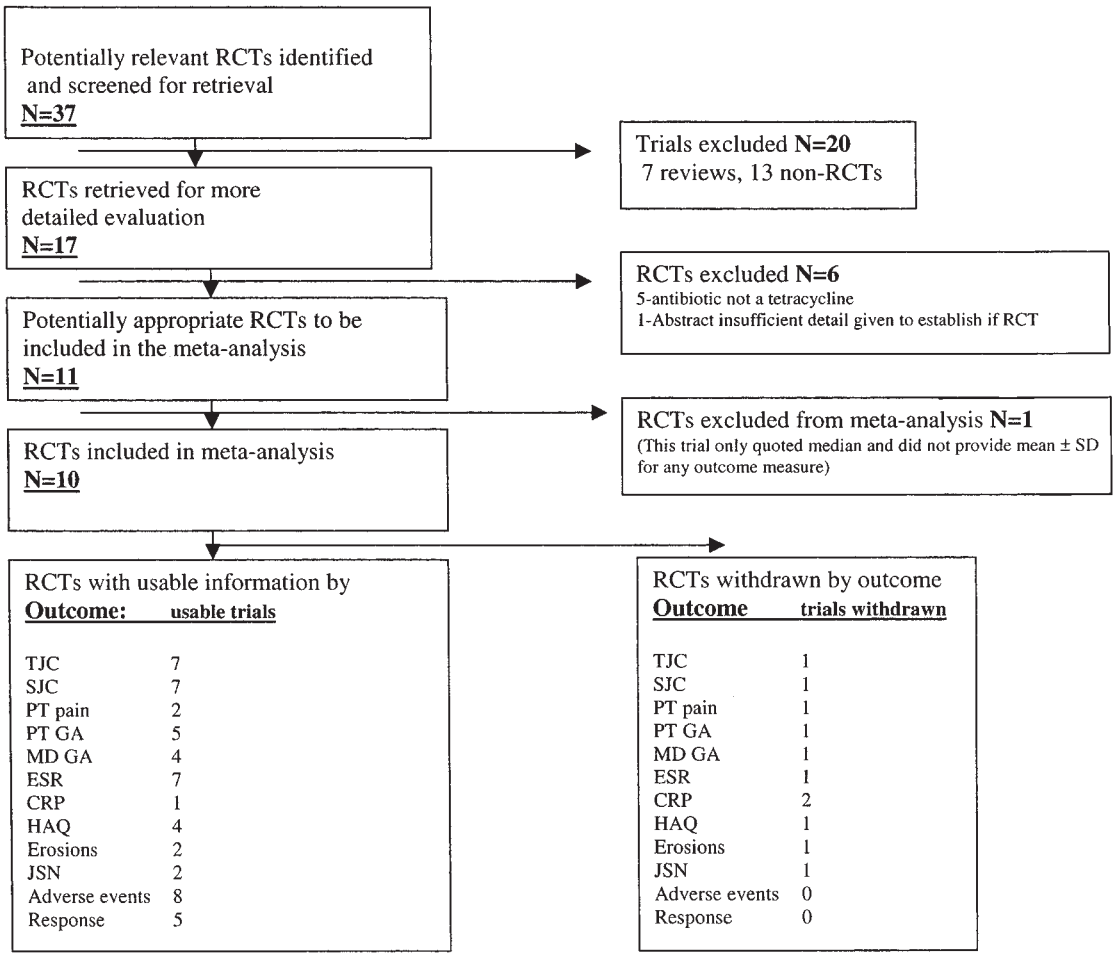

Figure 1. Trial flow diagram

groups. The intervention type and dose, treatment duration, and followup periods used in individual studies are presented in Table 2.

Methodological quality of studies. The methodological quality of the studies varied. Three studies satisfied all 4 criteria as defined above[27,30,31]. In the remaining studies elements of bias could not be excluded. In 3 studies, blinding of outcome assessors could not be determined[25,26,32]. Three studies were not analyzed using intention-to-treat methods[27,30,31]. In 2 studies[28,29] blinding of randomization was not clearly described, and in 3 studies blinding of intervention was not adequately described[12,28,29].

Quantitative data synthesis. Details on outcome measures reported in each study and the total numbers of patients reported for each outcome measure are given in Table 3. One study used only a median to present results and did not quote a mean ± SD[12]. Thus, it was not possible to combine the results statistically for continuous variables where this information was missing.

Disease activity. Eight studies reported on TJC and SJC (n = 535; Figure 2). Only 7 studies (505 subjects) could be combined statistically for these outcomes[25,26,28-30,32,33]. When all 7 studies were combined, there was a statistically significant benefit in favor of treatment for TJC: SMD (random effect model) = –0.39 with 95% confidence interval (CI) –0.74, –0.05; and for SJC, SMD (random effect model) = –0.23, 95% CI –0.41, –0.05 (Figures 2 and 3). Eight studies reported on ESR (n = 520). Only 7 studies (n = 490) could be combined statistically[25,26,28-30,32,33] (Figure 4). All studies reported a significant decrease in ESR in favor of treatment. The overall estimate of effect was strongly in favor of treatment, with WMD (random effect model) = –8.96, 95% CI –14.51, –3.42 when the studies were combined. No significant heterogeneity was noted by chi-square test, but the confidence interval for the Sreekanth study[28] barely overlapped that of several studies suggesting heterogeneity. Thus a random effects model was used in this case.

Three trials reported on CRP (n = 176 subjects). In one

Table 2. Interventions.

	Quality	"Intention-to-treat" Analyses	Antibiotic, mg/day	Control	Duration of Treatment, weeks
Skinner 1971	Poor	No	Tetracycline 250	Placebo daily	52
Kloppenburg 1994	Good	Yes	Minocycline 200	Placebo daily	26
Tilley 1995	Medium	Yes	Minocycline 100	Placebo daily	48
Kloppenburg 1996	Poor	No	Minocycline 200	Placebo daily	26
O'Dell 1997	Medium	Yes	Minocycline 200	Placebo daily	26
Bluhm 1997	Good	Yes	Minocycline 100	Placebo daily	48
Sreekanth 2000	Poor	No	Doxycycline 200	MTX, 7.5 mg/week	26
Van der Laan 2000	Poor	Yes	Doxycycline 50*	Placebo daily	36
St. Clair 2001	Good	Yes	Doxycycline 200**	Placebo or azithromycin	11
O'Dell 2001	Medium	Yes	Minocycline 100	HCQ 200 mg/day	104

Trial Kloppenburg 1996 is an extension of Kloppenburg 1994 and trial Bluhm 1997 is an extension of trial Tilley 1995. However, all trials report on separate outcomes so are listed as individual trials here, but patients are only counted once towards the overall number of patients included in the review. * mg/BID; ** mg IV.

Table 3. Outcome measures reported in studies.

		Outcomes											
Study	Patients	TJC	SJC	PT Pain	PT GA	MD GA	HAQ	Erosion	JSN	CRP	ESR	Response	Adverse Events
Skinner 1971	30	•	•								•		•
Kloppenburg 1994	80	•	•	•			•	•	•	•			•
Tilley 1995	219	•	•		•	•	•				•	•	
Kloppenburg 1996	65									•	•	•	•
O'Dell 1997	46	•	•		•	•					•	•	•
Bluhm 1997	219							•	•				
Sreekanth 2000	35	•	•		•	•	•				•		•
Van der Laan 2000	34	•	•		•						•		•
St. Clair 2001	31	•	•	•	•	•	•			•	•	•	•
O'Dell 2001	60	•	•	•	•	•	•				•	•*	•
Total	535	535	535	171	425	391	425	299	299	176	520	421	381

TJC: tender joint count, SJC: swollen joint count, PT Pain: patient global assessment of pain, PT GA: patient global assessment of disease activity, MD GA: physician global assessment of disease activity, HAQ: Health Assessment Questionnaire, JSN: joint space narrowing, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate. Trial Kloppenburg 1996 is an extension of Kloppenburg 1994 and trial Bluhm 1997 is an extention of trial Tilley 1995. However all trials report on separate outcomes so are listed as individual trials here, but patients are only counted once towards the overall number of patients included in the review. * The trial duration was 2 years but for this outcome the one-year response criterion (ACR 50) was available and was combined in the meta-analysis, see text for details.

trial[30] the data were not shown, but it was stated in the results that no significant difference at 28 days or 84 days was noted between treatment and control. With regard to the other trials, one[31] was an extension of the first[33] and described the same group of patients. There was a significant change in CRP in the treatment group (p = 0.0001), and there was a significant difference between groups in favor of treatment.

Six trials reported on PT GA (n = 425), but only 5 could be combined statistically[25,26,28,29,32]. Only one trial in the analysis showed a statistically favorable benefit toward treatment[25], with SMD –0.97, 95% CI –1.59, –0.36. When all the studies were combined, there was no significant benefit in favor of treatment: SMD = –0.15, 95% CI –0.036, 0.05. Five trials reported on MD GA, but only 4 of these could be combined statistically[25,26,28,32]. There was no significant combined overall reduction in MD GA when all the studies were combined: SMD (random effects model) = 0.00, 95% CI –0.7, 0.7. For MD GA only one trial showed an improvement in physician assessment of disease activity[25]. This study differed from the others in that it included a population of subjects with early onset disease in which all were seropositive.

Only 3 trials reported on patient self-reported pain levels (PT PAIN) (n = 171). One trial reported median values only[30]; therefore only 2 trials could be combined to estimate the overall effect size. Both studies reported an improvement in pain score in favor of therapy, and the overall estimate of effect was SMD = –0.68, 95% CI –1.03, –0.33.

Disease damage. Two studies reported on erosions (Figure 5) and/or JSN (n = 299 patients)[27,33]. Both studies treated all subjects for 48 weeks. Neither study showed a significant reduction in erosions or JSN. When the studies were combined, no statistically significant reduction in erosions was noted in favor of treatment: SMD (random effect model) = 0.17, 95% CI –0.29, 0.64; for JSN, SMD = 0.04, 95% CI –0.19, 0.27.

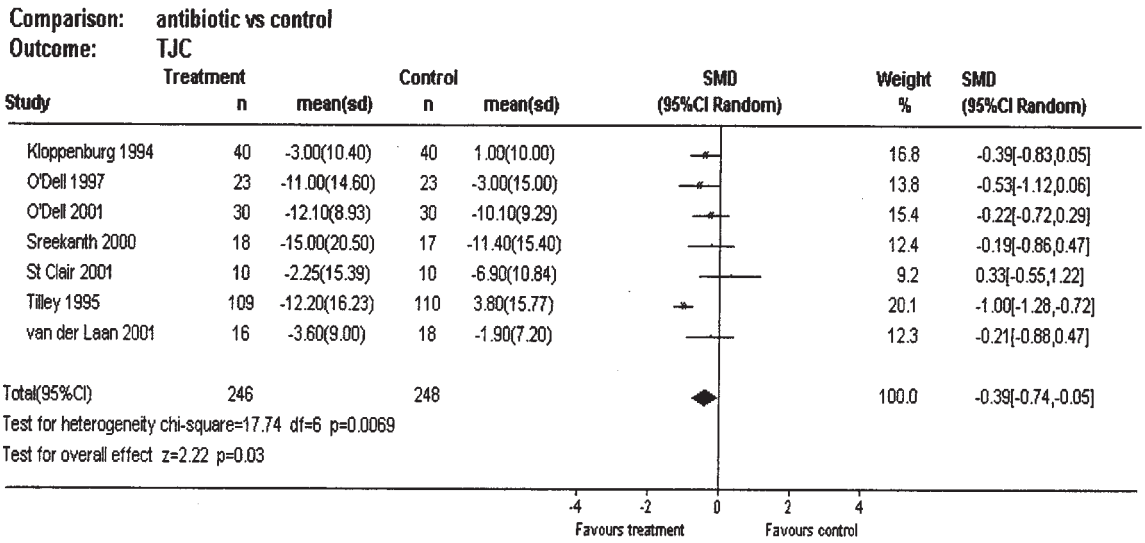

Figure 2. Metaanalysis of tender joint count (TJC) in patients with RA randomized to receive antibiotic or control treatment.

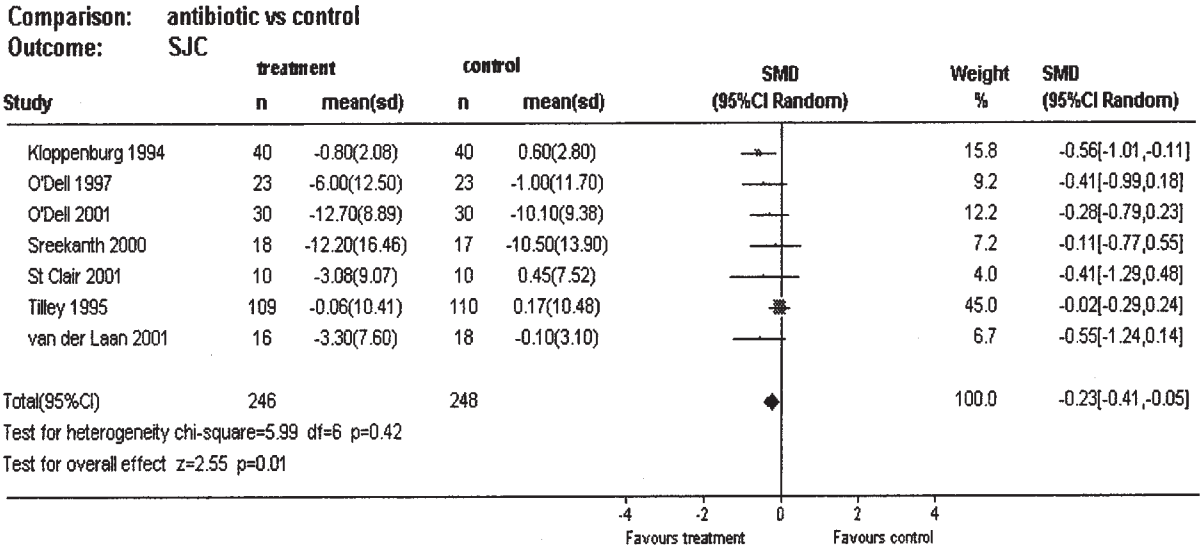

Figure 3. Metaanalysis of swollen joint count (SJC) in patients with RA randomized to receive antibiotic or control treatment.

Disability. Five studies reported on disability measured by HAQ. Only 4 studies could be combined in the analysis (n = 394)[26,28,32,33]. When combined in the analysis the overall estimate of effect favored treatment marginally: WMD = –0.15, 95% CI –0.28, –0.02.

Adverse effects. Eight studies reported on adverse effects, a total of 381 patients. When the studies were combined there was no statistical difference in the absolute risk of adverse effects between treatment and control groups, with absolute risk difference (ARD) (random effects model) = 0.10, 95% CI –0.01, 0.21. Unfortunately, the information available was inadequate to allow detailed analysis of individual side effects in the studies. However, a separate analysis was performed to determine if there was any difference between tetracyclines, but no difference between drugs was detected.

Response to treatment. Four studies defined response to treatment (total 421 subjects). Definitions of response differed in each study. However, all studies had more responders in the treatment group. Only 2 studies used the ACR criteria to define response to treatment[26,30]. The first reported an ACR 20 at days 28 and 84. The latter reported ACR 50 at year 1, which was the outcome included in this metaanalysis. However, ACR 20, ACR 50, and ACR 70 were also reported at year 2. Of these, only the ACR 50 at year 2 was significantly different between treatment groups (p = 0.04). When all the studies were combined the overall estimate of effect favored the treatment group, with relative risk (random effects model) = 1.78, 95% CI 1.0, 3.16.

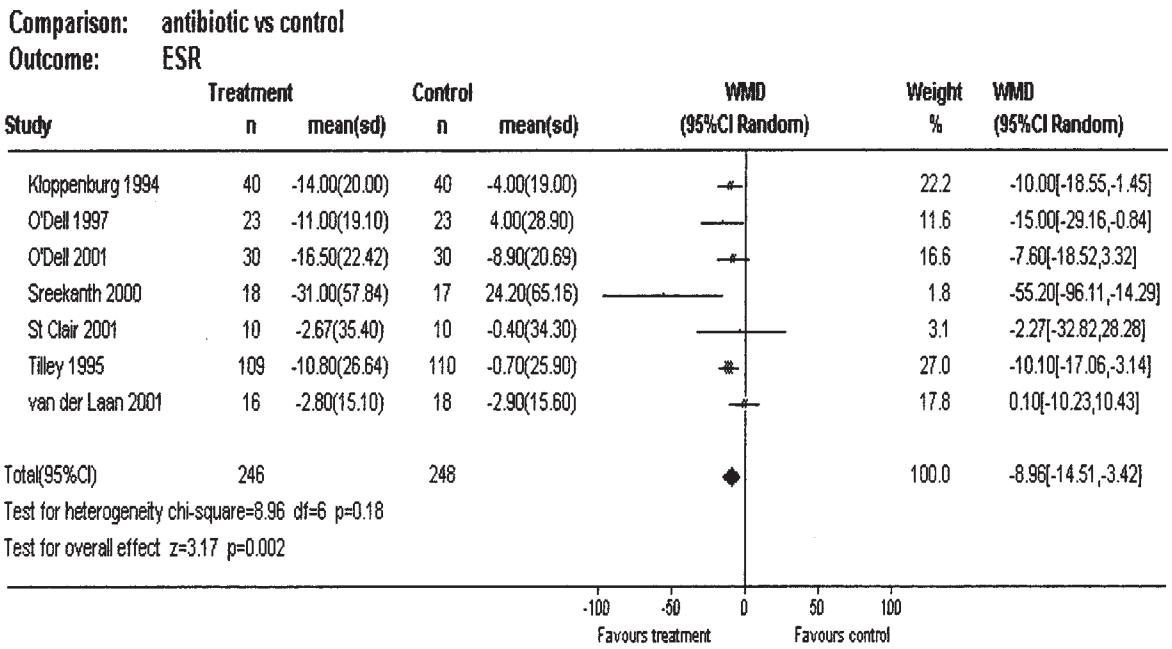

Figure 4. Metaanalysis of ESR in patients with RA randomized to receive antibiotic or control treatment.

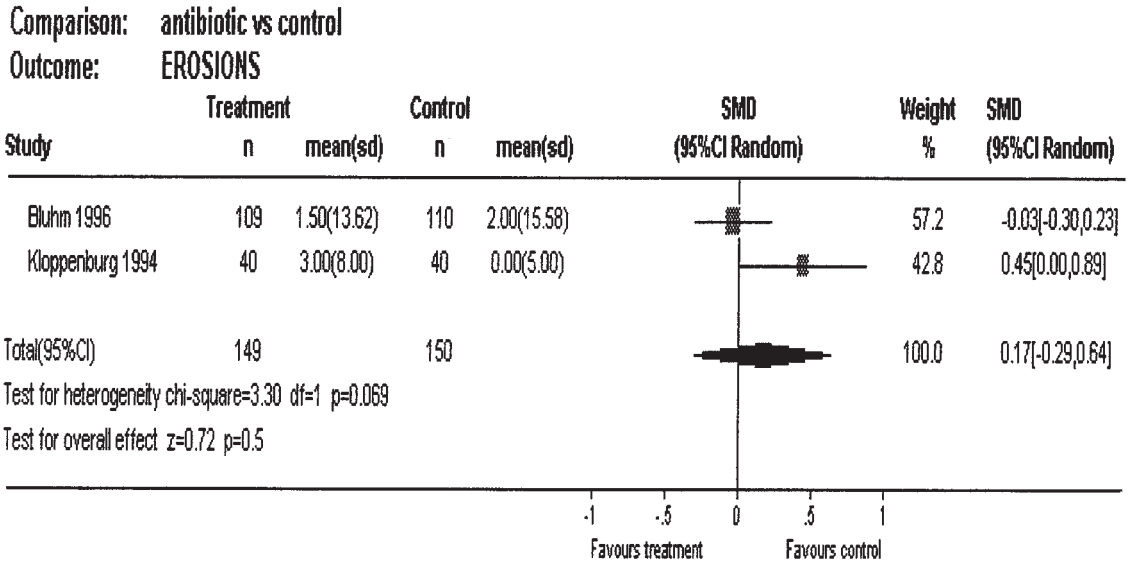

Figure 5. Metaanalysis of joint damage (erosions) in patients with RA randomized to receive antibiotic or control treatment.

Subgroup analysis excluding the cohort of early onset RA[25,26] showed a reduced but statistically significant benefit still favoring treatment, RR = 1.16, 95% CI 0.92, 1.46.

Subgroup analysis. The primary analyses included comparison of tetracycline antimicrobials to control (placebo or conventional treatment). A subgroup analysis was performed to test if the results were robust when comparison was made between antibiotic and placebo alone. This involved excluding 2 trials[26,28] for the following outcome measures: TJC, SJC, MD GA, PT PAIN, ESR, adverse events, and HAQ. One of the excluded trials[28] compared doxycycline to MTX, and the other compared minocycline to HCQ[26]. The results were robust, apart from one outcome measure, PT PAIN, where the previously noted improvement in the treatment group was lost after exclusion of the trials.

A second subgroup analysis was performed excluding the 3 trials that reported on doxycycline for the outcome measures TJC, SJC, and ESR[28-30]. Interestingly, the estimate of effect revealed a greater reduction in TJC and ESR in the treatment groups: TJC with SMD = –0.69, 95% CI –0.89, –0.49, and ESR with WMD = –10.14, 95% CI –14.72, –5.51 compared to the controls. No change was noted for SJC.

DISCUSSION

In this systematic review we included 10 RCT, a total of 535 subjects, describing at least one of the ACR core sets of outcome measures. This analysis illustrates that tetracyclines were associated with a reduction in disease activity with no absolute increased risk in adverse events compared to controls, but no statistically significant reduction in joint damage was noted.

The magnitude of reduction in disease activity was small for joint counts, but large for acute phase reactants when considering minocycline and doxycycline therapy. *In vitro* studies[15-20] indicate that these drugs have an important anti-inflammatory effect, in addition to their antibiotic properties, which is a likely explanation of the dramatic improvement in acute phase reactants in the absence of substantial clinical disease activity improvement.

The minimum effect size considered to be clinically meaningful in RA has been estimated by Kazis, *et al* to be 0.30[34]. Therefore the reductions in TJC and SJC represent small but nonetheless clinically meaningful effects. Further, moderate to substantial clinical benefit was observed for improvement in disease activity and acute phase reactants when subgroup analysis was performed on the minocycline trials alone. Apart from the work by Kazis, *et al* there is a paucity of literature on the translation of effect sizes into clinically relevant messages. The Cochrane database has published reviews on several of the commonly used DMARD for RA. The effect sizes reported in the reviews for HCQ[35] and intramuscular gold[36] were comparable to what we have described in this metaanalysis for the tetracycline antibiotics with respect to TJC, SJC, and ESR. However, the effect sizes that we report with tetracycline antibiotics for patient pain and patient global assessment are better than those reported for gold and HCQ. In addition, similar to our findings for the tetracyclines, the Cochrane reviews did not show a measurable benefit on radiographic progression of disease with either of these 2 agents. This suggests that the tetracycline antibiotics have at least a comparable clinical effect to these agents, and perhaps may have some additional benefit in terms of reduction of pain and improvement in patient global response.

In this metaanalysis we did not determine an improvement in disease damage (erosions or joint space narrowing). This may be attributed to the short duration of treatment and followup and the small number of patients evaluated for this outcome. In the trial by Kloppenburg, *et al*[33] the patients had long duration of disease, which may have contributed to the lack of significant radiographic improvement. Moreover, it is possible that the studies may not have been adequately powered to detect a significant difference between treatment and control groups. Controlled clinical trials have shown that measurable radiographic progression of joint damage may be moderated by leflunomide[37], MTX[1], SSZ[3,37], infliximab[1], and etanercept[6]. In all these studies, improvement of signs, symptoms, and function also occurred, but the patients were treated for one year or longer. Based on our findings in this systematic review no definitive comment can be made with respect to the effect of tetracyclines on disease damage and progression. Further studies should be performed that have sufficient power to detect a change in radiographic disease progression before these drugs are dismissed as potential DMARD.

Adverse events were reported for only 381 patients and there was no absolute increased risk between treatment and control groups. This is surprising, as most physicians will be aware that dizziness is a common side effect of minocycline. It is possible that the relatively younger age of the patients included in some of the studies made this less of a problem. In addition, the risk of systemic lupus erythematosus and hepatotoxicity are relatively rare. True to life studies are clearly needed to evaluate these issues more carefully.

Only 2 of the trials used the standard definition of response to treatment (ACR 20) as a primary outcome measure, so it is difficult to compare the overall response rate with the new biologic agents or MTX, which both show substantial reductions in ACR 20, with treatment over 6 and 12 month periods for infliximab[1,5] and etanercept[6,38]. Using the definitions of response quoted by the authors[30,32,33], the overall response rate was higher in the treatment group. However, in one of the trials[25], minocycline is compared to HCQ. This was a 2 year study; however, the authors have reported an ACR 50 after 12 months of treatment in addition to the 2-year data. We combined the reported one-year ACR results in the metaanalysis rather than the 2-year data to avoid any potential bias in favor of treatment versus control, given that the remainder of the trials were less than one-year duration. At one year, the group achieved an ACR 50. Interestingly, at the 2-year point, 60% of the minocycline treated group compared to 33% in the HCQ group still satisfied the criteria for ACR 50 improvement. The subjects in this study all had early RA and were seropositive. In the trial by Moreland, *et al*, where etanercept was compared to placebo over a 6-month period[38], the ACR 50 at 6 months was 51% in those treated with etanercept and 17% in the placebo group. We recognize that there are some methodological issues involved in comparing response rates across trials when no direct comparisons between agents have been made. Therefore, without direct comparisons no definitive comment can be made with respect to the efficacy of one agent over another. However, based on our findings, it is

possible that the tetracyclines would compare favorably with the newer DMARD for RA in terms of efficacy, side effect profile, patient preference, and cost-effectiveness.

An obvious limitation of this systematic review was the small number and low quality overall of the clinical trials available for review. In addition, we were unable in certain instances to combine all the included studies in the meta-analysis due to incomplete data. However, where possible a description of trial results was given to minimize any potential bias that may arise from exclusion of certain trials. In evaluating the quality of the trials we looked at 4 key criteria and subsequently categorized the studies as good, medium, or poor depending on the number of criteria each study satisfied. We adopted this qualitative approach rather than assigning a specific score to individual trials in order to simplify the overall message with regard to trial quality. In the past, authors have questioned the validity of using a quality score to categorize studies as good or bad[39], as important methodologic detail may be omitted from published reports. The quality of reporting is used as a proxy measure for methodologic quality of trials, but reporting may hide important differences in methodologic quality. Thus, there is a risk that well conducted trials may be reported badly. We acknowledge that this may account in part for the overall low trial quality reported here.

There was significant study heterogeneity between the trials for several outcome measures, which may have diluted the treatment effect. To detect study heterogeneity we used the chi-square test with a $p < 0.1$. However, given that we combined different types of tetracyclines using studies of different duration and with different patient characteristics, this warranted the use of the random effect model, regardless in many instances of the chi-square test results. The chi-square test is considered to be a rather liberal test and even with a p value < 0.1 some clear heterogeneity can be overlooked.

The primary objective of this review was to compare tetracycline treatment with control, which included either placebo or conventional therapy for RA. We decided to include the trials where there was an active comparator, as in recent years it is considered unacceptable practice to prescribe placebo alone to patients with RA. Therefore, excluding those trials that used an active comparator would have limited the review to studies published several years ago and merely replicated what previous authors have reported[40]. Since the publication of that review several well designed trials on the use of tetracyclines in RA have been published. Further, practice guidelines have changed; therefore studies comparing tetracycline therapy with placebo would no longer be justified for ethical reasons[41]. To overcome this potential limitation we performed a subgroup analysis excluding those trials where the control was an active comparator. This subgroup analysis did not change the overall results significantly.

Several interesting observations emerged from this systematic review that are worthy of elaboration.

First, we found that patients with early onset disease had a better response to tetracycline antibiotics. This is not surprising, as it is recognized that patients with RA treated early after disease onset have a better prognosis. Eighteen percent of the patients included in the metaanalysis were exclusively seropositive and had early onset disease. Subgroup analysis excluding this cohort[25] of patients[26] was performed. This showed that the benefit related to treatment persisted, but was reduced. Seropositivity has been shown to correlate with a poorer prognosis in RA. The remaining trials did have a high percentage of seropositive subjects in both treatment and control groups as well (Table 1). Due to small sample size, however, we were unable to do meaningful subgroup analysis based on degree of seropositivity in treatment and control groups at baseline.

Second, this metaanalysis showed that minocycline was more effective than doxycycline in reducing disease activity in RA. Some authors have suggested that doxycycline is less effective for RA than minocycline[29]. When subgroup analysis was performed excluding doxycycline, there was indeed a larger reduction in disease activity, shown by further improvement in TJC and ESR. This suggests that in the tetracycline family of antibiotics, minocycline may be more effective in reducing disease activity. However, there has been no direct comparison between the individual tetracyclines in any of the studies to date, thus a definitive statement on the superior efficacy of one over the other at this point cannot be made.

In our metaanalysis of tetracycline antibiotics only one trial reported subjects followed for more than one year[26], therefore, we cannot draw any conclusions on the longterm efficacy of tetracycline therapy for RA. However, in this study the patients were followed for 2 years, and ACR 50 at 2 years was significantly better in the treatment group compared to controls. However, radiographic outcomes were not reported in the results of this trial. Radiological outcomes may be a more important indicator of biological response to treatment than improvement in ACR core variables alone[42]. In addition, ACR criteria for response may be insensitive to change in early onset disease where disease may be mild or in evolution. Indeed, demonstrating improvement in these criteria may have no bearing on longterm outcome[43]. Future studies looking at the longterm results of tetracycline therapy in RA should therefore include radiographic outcomes.

In summary, this is the first metaanalysis that reports on tetracycline antibiotics for RA, and it demonstrates that tetracyclines when administered for more than 3 months lead to a reduction in disease activity and acute phase reactants in RA. The effect is most marked for minocycline. In addition, the treatment effect is more marked in the subgroup of patients with short disease duration (less than

one year) who were also seropositive. With established efficacy proven for methotrexate, HCQ, sulfasalazine, infliximab, etanercept, and leflunomide in RA, is there a role for the tetracyclines? We suggest that these drugs do have a potentially important role, which may have been overlooked with the advent of newer agents. They have been shown to be safe and effective at least in the short-term. Indeed, they may prove to be a safer and more cost-effective alternative to some of the newer biologic agents, although studies are required to determine their side effect profile. Further study is warranted to compare the tetracyclines to some of the newer DMARD, including the biologics. Finally, the use of tetracyclines in combination with some of these other DMARD may have additive or multiplicative effects. This should also be formally tested in a randomized controlled trial.

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