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Combination Antibiotics as a Treatment for Chronic *Chlamydia*-Induced Reactive Arthritis

A Double-Blind, Placebo-Controlled, Prospective Trial

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Objective. ***Chlamydia trachomatis* and *Chlamydophila (Chlamydia) pneumoniae* are known triggers of reactive arthritis (ReA) and exist in a persistent metabolically active infection state in the synovium, suggesting that they may be susceptible to antimicrobial agents. The goal of this study was to investigate whether a 6-month course of combination antibiotics is an effective treatment for patients with chronic *Chlamydia*-induced ReA.**

Methods. **This study was a 9-month, prospective, double-blind, triple-placebo trial assessing a 6-month course of combination antibiotics as a treatment for *Chlamydia*-induced ReA. Eligible patients had to be positive for *C trachomatis* or *C pneumoniae* by polymerase chain reaction (PCR). Groups received 1) doxycycline and rifampin plus placebo instead of azithromycin; 2) azithromycin and rifampin plus placebo instead of doxycycline; or 3) placebos instead of azithromycin, doxycycline, and rifampin. The primary end point was the number of patients who improved by 20% or more in at least 4 of 6 variables without worsening in any 1 variable in both combination antibiotic groups combined and in the placebo group at month 6 compared with baseline.**

Results. **The primary end point was achieved in 17 of 27 patients (63%) receiving combination antibiotics and in 3 of 15 patients (20%) receiving placebo. Secondary efficacy end points showed similar results. Six of 27 patients (22%) randomized to combination antibiotics believed that their disease went into complete remission during the trial, whereas no patient in the placebo arm achieved remission. Significantly more patients in the active treatment group became negative for *C trachomatis* or *C pneumoniae* by PCR at month 6. Adverse events were mild, with no significant differences between the groups.**

Conclusion. **These data suggest that a 6-month course of combination antibiotics is an effective treatment for chronic *Chlamydia*-induced ReA.**

Chlamydia trachomatis and various species in the genera *Salmonella, Shigella, Campylobacter*, and *Yersinia* are all known triggers of reactive arthritis (ReA) (1). A number of studies have also indicated that *Chlamydophila (Chlamydia) pneumoniae* is another, although less frequent, causative agent in ReA (2,3). *C trachomatis* and *C pneumoniae* are both commonly acquired asymptomatically (4,5), making the causative trigger less clinically apparent in many cases (6). Polymerase chain reaction (PCR) technology occasionally has demonstrated the presence of chromosomal DNA from the known triggering organisms in the synovial tissue of patients with the postdysentery form of ReA (7–10). This same technology has demonstrated the routine presence of both *C trachomatis* and *C pneumoniae* DNA

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in synovial tissue from patients with postchlamydial arthritis (11–13). One important difference is that these chlamydial species exist in a persistent metabolically active infection state, whereas the postenteric organisms do not (with the possible exception of *Yersinia* [14]), suggesting that persistent *Chlamydia* may be uniquely susceptible to antimicrobial agents.

Persistent chlamydial organisms exist in a morphologically aberrant, but metabolically active, state in synovial tissue (12,15). The pattern of gene expression is significantly different in the persistent infection state as compared with the pattern characteristic of normal active infection. During persistence, expression of the major outer membrane protein gene (*Omp1*) and several genes required for the cell division process is severely down-regulated. This is coupled with differential up-regulation of the 3 *C trachomatis* paralog genes encoding Hsp60 (*Ct110*, *Ct604*, and *Ct755*) (16).

The use of long-term antibiotic treatment for patients with ReA is controversial. Several reported studies have indicated that prolonged antimicrobial monotherapy is not efficacious (17–20). Indeed, in vitro data confirm these findings (21–24). Yet, other studies suggest there might be a benefit, specifically with *Chlamydia*-induced ReA (25,26), and that this treatment approach might also work better in early disease (27). Conversely, a recent study suggested no benefit with a 4-month course of doxycycline in *Chlamydia*-induced ReA (28). Rifampin has excellent tissue penetration, which is mandatory when treating obligate intracellular pathogens such as *Chlamydia*. Rifampin also has been shown to attenuate all chlamydial gene transcription, including the heat-shock proteins (HSPs) (29). The HSPs may prime the infected cell for eradication (30,31), allow for proper apoptosis (32,33), and/or eliminate the immunogenic source (34). Combining this effect with antibiotics that block chlamydial protein synthesis (e.g., doxycycline or azithromycin) may allow for successful eradication of the cell harboring persistently infecting intracellular organisms. Interestingly, the same in vitro data cited above suggest synergistic eradication of the persistent chlamydial infection with a combination of azithromycin and rifampin (22).

A recent pilot study conducted by our group suggested that prolonged treatment with a combination of doxycycline and rifampin significantly improves symptoms of chronic undifferentiated spondylarthritis (SpA) (with a special focus on *Chlamydia*) compared with doxycycline alone (35). The goal of the present study was to further investigate whether a 6-month course of combination antibiotics, one of which is rifampin, is effective in the treatment of patients with chronic *Chlamydia*-induced ReA.

PATIENTS AND METHODS

Patients. Eligible patients were ages 18–70 years, fulfilled the European Spondylarthropathy Study Group (ESSG) preliminary criteria for the classification of SpA (36), which were modified as described below, and had disease duration of $\geq$6 months. Patients were excluded if they had current psoriasis, a history of ankylosing spondylitis (AS) or inflammatory bowel disease (IBD), previous exposure to antibiotics (>2 weeks) as a potential treatment for their ReA, or a history of sensitivity or allergic reaction to rifampin, doxycycline, or azithromycin. Other standard exclusions applied. Those receiving concurrent treatment with disease-modifying antirheumatic drugs (DMARDs) or biologic agents were not excluded.

For this study, the ESSG criteria were modified as inclusion criteria to increase the likelihood of specifically recruiting patients with postchlamydial ReA. The ESSG criteria include a past or present diagnosis of IBD and a present diagnosis of psoriasis. We used these as exclusion criteria since we did not want to enroll patients with IBD-related SpA or patients with psoriatic arthritis. Another ESSG criterion is an episode of acute diarrhea occurring within 1 month before arthritis. This was also used as an exclusion criterion in order to eliminate patients with postdysentery ReA. The ESSG inclusion criteria were otherwise unchanged.

Study design. This study was a 9-month, prospective, double-blind, triple-placebo trial conducted at 4 centers in the US and Canada. All participating sites received approval from their governing institutional review board (or equivalent); all patients provided informed written consent.

Individuals were recruited and screened from April 2006 through December 2007. At screening, their medical history was recorded, and the modified ESSG criteria were carefully reviewed. Other information recorded included results of a complete physical examination, swollen joint count (SJC), and tender joint count (TJC), responses to a questionnaire regarding the duration and severity of their low back and peripheral joint pain and stiffness, score on the Health Assessment Questionnaire (HAQ) disability index (DI) (37), HLA–B27 status, and a history of any known chlamydial exposure (to both *C trachomatis* and *C pneumoniae*) with detailed timing of that event. Because of the study population, a previously established scoring system was used that included 76 swollen joints and 78 tender joints (to include the distal interphalangeal and carpometacarpal joints) (38). A second modified SJC was also recorded at all visits; this modified 76–swollen joint count included dactylitis. Any subject with dactylitis had that added to the SJC; 1 swollen digit was counted as equal to 1 swollen joint (this digit, however, was not counted twice). Dactylitis was not quantified but was recorded in a qualitative manner only. The principal investigator reviewed any recent pelvic or dedicated sacroiliac radiographs to determine the presence of sacroiliitis.

A blood sample was obtained from all subjects at the screening visit and shipped at ambient temperature via overnight courier from the clinical sites to the laboratory of one of

us (APH). In patients with active synovitis who consented to the procedure, synovial tissue was obtained at the same time by blind synovial biopsy using a Parker-Pearson needle (39). The site biopsied in all subjects was the knee, with the exception of 1 sample obtained from the wrist by open surgical procedure. These samples were immediately snap-frozen in liquid nitrogen and stored at –80°C until shipping on dry ice by overnight courier to author APH's laboratory.

At APH's laboratory, PCR was used to assess chlamydial DNA in peripheral blood mononuclear cells (PBMCs) or synovial tissue, and these assays targeting *C trachomatis* and *C pneumoniae* have been described extensively (12,13,15,40,41). Screening PCR assays were performed in duplicate by each of 2 individuals (JAS and CO), and standard analyses included assays targeting at least 2 different DNA sequences from each organism. The *C trachomatis*–directed assays targeted *Omp1* and the 16S ribosomal RNA genes. Assays to determine the presence of *C pneumoniae* DNA targeted the homologous genes in that organism; *C trachomatis*–directed primer systems do not amplify sequences in *C pneumoniae* and vice versa. Samples were considered positive if both duplicates in all assays matched. In the case of discrepancies, a third set of assays targeting several other genes was used for resolution. Extreme care was taken to avoid contamination of PCR-related materials.

Treatment and testing. Those subjects who were positive by PCR for either chlamydial species in PBMCs and/or synovial tissue were randomly assigned (1:1:1) to 1 of 3 treatment groups; randomization was stratified by age ($<$40 years or $\geq$40 years) and disease duration (0.5–2 years or $>$2 years) in order to achieve a more homogeneous patient population in each of the 3 groups. The first group received 100 mg doxycycline by mouth twice daily and 300 mg rifampin by mouth daily plus placebo instead of azithromycin; the second group received 500 mg azithromycin by mouth daily for 5 days and then 500 mg azithromycin by mouth twice weekly and 300 mg rifampin by mouth daily plus placebo instead of doxycycline; the third group received 3 placebos instead of azithromycin, doxycycline, and rifampin. All 3 groups were treated for a total of 6 months and followed up for 3 months after completion of the study drugs (total of 9 months). The purpose of the month 9 visit was to determine whether the patient's condition had worsened after discontinuing study medications.

In addition to the study drugs, patients were allowed to take oral corticosteroids ($\leq$10 mg/day prednisone or equivalent) and/or nonsteroidal antiinflammatory drugs (NSAIDs) if they had been receiving stable doses for $>$4 weeks prior to randomization; DMARDs and biologic agents were allowed if subjects had been receiving stable doses $>$12 weeks prior to randomization. The doses of these medications could not be increased; however, if the subject experienced clinical improvement during the study, downward adjustment of these background medications was allowed.

Study subjects were evaluated at screening, baseline, and then at months 1, 3, 6, and 9. *C trachomatis* and *C pneumoniae* serum titers were checked at baseline, but were not rechecked at any time point during the study. Urogenital chlamydial testing was not performed. Disease activity assessments were performed at each study visit. Study subjects were queried at each visit as to whether they believed their disease was in remission. Disease remission was a strict, but subjective, determination requiring patients to state their belief that their disease-related symptoms were "100% resolved." The erythrocyte sedimentation rate (ESR) was measured at each visit, and the level of high-sensitivity C-reactive protein (hsCRP) was measured at baseline and at months 1, 3, and 6. Safety was assessed routinely at each visit, including completion of an adverse event (AE) form. AEs were predefined as any new medical diagnosis or condition; serious AEs were predefined as AEs that included hospitalization, administration of intravenous antibiotics, or death.

PCR results were followed up in a blinded manner throughout the study. All randomized subjects had a PBMC PCR for each chlamydial species repeated at months 1, 6, and 9 in addition to initial screening. Subject to consent, each patient whose synovial tissue PCR at screening showed positive results underwent a repeat synovial biopsy of the same joint at month 6.

Statistical analysis. We estimated that we would require a total sample size of 42 subjects to obtain 80% power to detect a 73% improvement in the groups receiving combination antibiotics and a 20% improvement in the placebo group at a significance level of 0.05. Of the patients who received combination antibiotics in our pilot trial, 73% were considered responders; only 13% in the monotherapy group responded (35). Since the patient in the pilot trial was not blinded to the therapy, we increased the expected placebo response rate in this study. Patients were randomized in a 2:1 manner (combination antibiotics versus placebo). The study was not powered to detect a difference in efficacy between the antibiotic combinations in the 2 groups.

The primary end point in this study was the number of patients who improved by 20% or more in at least 4 of 6 variables without worsening in any 1 variable (responders) in both combination antibiotic groups combined and in the placebo group at month 6 compared with baseline. These 6 variables included a modified 76–swollen joint count (as described above), a 78–tender joint count, and responses on a 4-component questionnaire (average duration of morning stiffness in the low back per day over the past week, current low back pain on a 0–100-mm visual analog scale [VAS], current peripheral joint pain on a 0–100-mm VAS, and global assessment of disease activity on a 0–100-mm VAS). This questionnaire was used in previous ReA studies (35,42).

Efficacy and safety analyses were performed on an intent-to-treat (ITT) basis. Subjects who prematurely withdrew or who were lost to followup for any reason were included in the ITT population and were considered nonresponders. For the primary efficacy analysis, Fisher's exact test 2×2 contingency table was used to compare the difference at month 6 between responders receiving combination antibiotics and those receiving placebo.

Baseline demographics of the study patients were analyzed using Fisher's exact test 2×2 contingency table or unpaired *t*-tests, depending on whether the data were categorical or quantifiable. The secondary efficacy analyses included each component of the responder determination in an independent manner; other secondary efficacy analyses included the physician's global assessment of disease activity on a 0–100 mm VAS, as well as the ESR, hsCRP level, and HAQ DI score. These were analyzed using unpaired *t*-tests. Remission rates

Table 1. Baseline characteristics and demographics of the study subjects*

Characteristic	Combination antibiotics (n = 27)	Placebo (n = 15)
Age, years	44.2 ± 12.3	49.0 ± 16.4
Men, no. (%)	15 (56)	9 (60)
Race, no. (%)		
Caucasian	18	9
African American	9	5
Other	–	1
Disease duration, years	10.4 ± 12.1	14.2 ± 14.2
SJC, 0–76 joints	3.4 ± 2.4	3.8 ± 2.7
TJC, 0–78 joints	5.0 ± 4.3	7.9 ± 7.4
Duration of morning low back stiffness, hours	1.7 ± 1.4	1.0 ± 0.9
Patient's global assessment of disease activity, 0–100-mm VAS†	44.9 ± 23.2	40.3 ± 22.9
Physician's global assessment of disease activity, 0–100-mm VAS†	59.9 ± 12.8	61.6 ± 15.8
HAQ DI score	0.77 ± 0.46	0.93 ± 0.61
Axial arthritis, no. (%)	20 (74)	12 (80)
Peripheral arthritis, no. (%)	26 (96)	13 (87)
Active enthesitis, no. (%)	17 (63)	9 (60)
Active dactylitis, no. (%)	7 (26)	6 (40)
Active iritis, no. (%)	3 (11)	0 (0)
History of iritis, no. (%)	7 (26)	0 (0)
Active KB, no. (%)	3 (11)	0 (0)
History of KB, no. (%)	6 (22)	3 (20)
Active CB, no. (%)	0/15 (0)	1/9 (11)
History of CB, no. (%)	2/15 (13)	2/9 (22)
Active urethritis, no. (%)	2 (7)	1 (7)
History of *Chlamydia trachomatis* at any time point, no. (%)	14 (52)	5 (33)
History of *C trachomatis* within 1 month of arthritis, no. (%)	3 (11)	1 (7)
Known history of *C pneumoniae* infection, no. (%)	0 (0)	0 (0)
Use of NSAIDs, no. (%)	20 (74)	11 (73)
Use of corticosteroids, no. (%)	4 (15)	0 (0)
Use of DMARDs, no. (%)‡	7 (26)	3 (20)
Radiographic sacroiliitis, no. (%)§	19/20 (95)	8/10 (80)
HLA–B27 positive, no. (%)	11/24 (46)	3/13 (23)

* Except where indicated otherwise, values are the mean ± SD. The only significant difference between the groups was for a history of iritis ($P = 0.04$). SJC = swollen joint count; TJC = tender joint count; VAS = visual analog scale; HAQ DI = Health Assessment Questionnaire disability index; KB = keratoderma blennorrhagicum; CB = circinate balanitis; NSAIDs = nonsteroidal antiinflammatory drugs.
† 0 = best; 100 = worst.
‡ Disease-modifying antirheumatic drugs (DMARDs) used in the combination antibiotics group included sulfasalazine (n = 6) and methotrexate (n = 1). DMARDs used in the placebo group included sulfasalazine (n = 1), methotrexate (n = 1), and hydroxychloroquine (n = 1).
§ Defined as asymmetric appearance with at least grade II unilateral changes. Routine radiography of the sacroiliac joints was not part of the study protocol. These data were only collected if the study subjects had previous radiography performed as part of their routine clinical care. Thirty of the 42 subjects (71%) had previous radiographs of their sacroiliac joints available for review.

and PCR results were analyzed in a manner similar to analysis of the primary end point using Fisher's exact test.

RESULTS

Characteristics of the study patients. Study data were collected between April 2006 and October 2008. A total of 80 subjects were screened, and 42 were enrolled and randomized to treatment. Twelve subjects were randomized to doxycycline and rifampin, 15 were randomized to azithromycin and rifampin, and 15 were randomized to matching oral placebos. Table 1 summarizes the demographics and baseline characteristics of subjects receiving combination antibiotics and those receiving placebo. More men than women were enrolled, and the mean age ranged from 44.2 years to 49 years. These subjects had chronic disease, with a mean disease duration >10 years. The only baseline characteristic that differed significantly between study groups was that subjects randomized to combination antibiotics were more likely to have a history of iritis. The majority of randomized subjects had positive serologic results for *Chlamydia* at baseline. Specifically, 35 of 42, 9 of 42, 2 of

42, and 1 of 42 subjects were positive for *C pneumoniae* IgG, *C pneumoniae* IgA, *C trachomatis* IgG, and *C trachomatis* IgA, respectively.

The demographics and baseline characteristics of all 80 subjects who were screened were similar to those of the 42 who were enrolled and randomized. Specifically, their mean age was 45.5 years (range 21–70 years) with mean disease duration of 9.9 years (range 0.5–40 years). Comparing baseline demographics and characteristics of the 2 combination antibiotic groups also yielded similar results. There were no statistically significant differences between the 2 groups that received active treatment (data not shown).

Treatment. All randomized study patients received at least 1 dose of the study drugs and were included in the ITT and safety analyses. Thirty-four of 42 subjects (81%) completed the 6 months of treatment; 3 of 27 subjects (11%) receiving combination antibiotics and 5 of 15 subjects (33%) receiving placebo discontinued the study medications prematurely. Of the 3 subjects receiving combination antibiotics who discontinued prematurely, 2 were receiving doxycycline and rifampin (reasons for discontinuation were peripheral edema and loss to followup), and 1 was receiving azithromycin and rifampin (reason for discontinuation was loss to followup). Regarding the 5 subjects receiving placebo, the reasons for discontinuation were lack of efficacy (n = 1), nausea and diarrhea (n = 1), seizures (n = 1), withdrawal of consent (n = 1), and loss to followup (n = 1).

Efficacy. The primary end point was achieved in 17 of 27 patients (63%) receiving combination antibiotics and in 3 of 15 (20%) receiving placebo ($P = 0.01$). Five of the 6 individual components that determined responder status improved significantly at 6 months compared with baseline in the combination antibiotic group ($P < 0.0001$, $P < 0.0001$, $P = 0.002$, $P = 0.01$, and $P = 0.008$ for the modified SJC, the TJC, current low back pain on a VAS, current peripheral joint pain on a VAS, and global assessment of disease activity on a VAS, respectively). The sixth component (average duration of morning stiffness in low back) revealed a trend toward improvement at 6 months compared with baseline in those subjects receiving combination antibiotics ($P = 0.068$). In the placebo group, none of the 6 components improved significantly at month 6 compared with baseline. Responders did not differ from nonresponders in their baseline characteristics, including age, sex, disease duration, axial and/or peripheral joint involvement, history of known chlamydial infections, or HLA–B27 status (data not shown). In a head-to-head comparison of the modified SJC and TJC in subjects receiving combination antibiotics and those receiving placebo, the modified SJC and TJC were significantly lower at month 1 and at all subsequent time points in subjects receiving combination antibiotics (Figure 1).

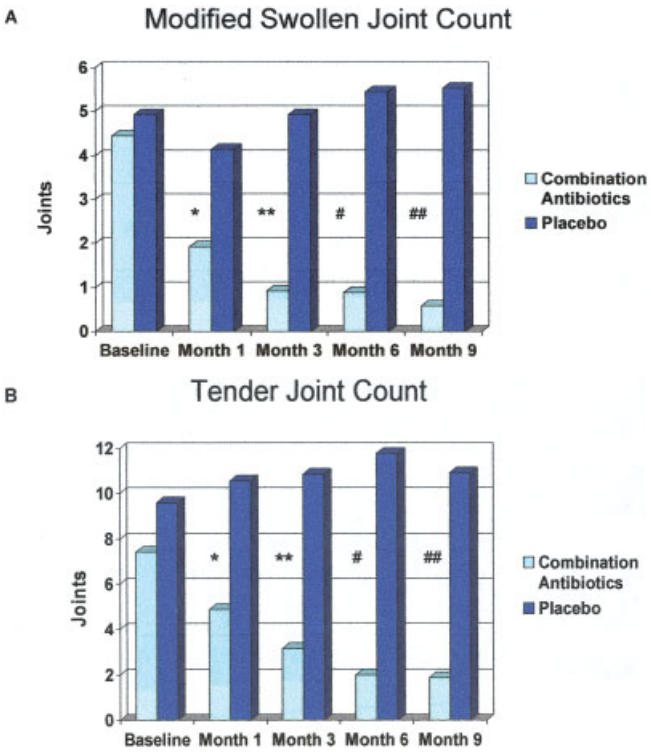

Figure 1. A, Modified swollen joint counts in patients receiving combination antibiotics compared with those receiving placebo. * = $P = 0.0001$; ** = $P < 0.0001$; # = $P = 0.0007$; ## = $P = 0.0005$, versus baseline. **B,** Tender joint counts in patients receiving combination antibiotics compared with those receiving placebo. * = $P = 0.0009$; ** = $P < 0.0001$; # = $P = 0.002$; ## = $P = 0.0004$, versus baseline. Values are the mean.

All secondary end points demonstrated either statistically significant improvement or a trend toward improvement in patients receiving combination antibiotics compared with those receiving placebo (Table 2). We found no significant worsening in any of the end points from month 6 to month 9. Seven of 27 subjects (26%) randomized to combination antibiotics and 4 of 15 subjects (27%) randomized to placebo had dactylitis at baseline; all 7 subjects receiving combination antibiotics had resolution of their dactylitis at month 6, while only 2 of 4 subjects receiving placebo experienced resolution. Downward adjustments in background DMARD usage were allowed if the subject reported feeling better. Four of the 7 subjects taking DMARDs at baseline in the

Table 2. Secondary end points*

Group (n), time of sampling	Physician's global assessment of disease activity, 0–100-mm VAS	HAQ DI score	ESR, mm/hour	hsCRP, mg/liter
Combination antibiotics (27)				
Baseline	63.9	0.84	25.1	1.07
Month 1	35.2	0.79	17.8	0.56
Month 3	23.2	0.68	17.7	0.63
Month 6	16.2	0.71	12.7	0.41
Month 9	17.7	0.57	14.0	ND
Placebo (15)				
Baseline	60.2	1.1	18.9	0.42
Month 1	52.9	0.92	25.2	0.27
Month 3	49.4	0.87	19.8	0.55
Month 6	45.7	0.99	17	0.34
Month 9	43.8	0.92	18.4	ND
P, combination antibiotics vs. placebo at month 6	0.0009	0.19	0.07	0.11
P, combination antibiotics vs. placebo at month 9	0.0003	0.13	0.02	–

* VAS = visual analog scale; HAQ DI = Health Assessment Questionnaire disability index; ESR = erythrocyte sedimentation rate; hsCRP = high-sensitivity C-reactive protein; ND = not done.

combination antibiotic group discontinued their DMARD during the trial (all 4 were taking sulfasalazine; 2 discontinued at month 1, 1 at month 3, and 1 at month 6); none of the 3 subjects in the placebo group who were taking DMARDs discontinued their background DMARD because of clinical improvement. Four subjects randomized to active treatment had been receiving long-term corticosteroids at baseline; 1 discontinued use, 1 decreased the daily usage, and the other 2 continued receiving a stable dose throughout the trial.

A final secondary end point followed up was remission. Six of 27 patients (22%) randomized to combination antibiotics believed that their disease went into complete remission during the trial, whereas no patient in the placebo arm achieved remission (P = 0.07). All 6 subjects who achieved remission did so by month 3 or month 6, and all continued to have remission of disease at month 9. The average disease duration of the 6 subjects who achieved remission was 8.5 years. Five of the 6 subjects who achieved remission were randomized to the azithromycin and rifampin group.

A post hoc analysis was performed to determine the number of patients who improved by 50% or more (50% responders) and by 70% or more (70% responders) in at least 4 of the 6 same variables that were used to determine responders. Of the 27 subjects randomized to combination antibiotics, 11 (41%) were 50% responders and 7 (26%) were 70% responders. Five of 15 subjects randomized to azithromycin and rifampin and 2 of 12 subjects randomized to doxycycline and rifampin were 70% responders. Five of the 6 subjects who believed that their disease went into remission were 70% responders. Regarding the 3 responders in the placebo group, 1 of 15 (7%) was a 70% responder.

PCR analyses. All patients had to be positive for either *C trachomatis* or *C pneumoniae* by PCR in PBMCs or synovial tissue in order to be randomized to treatment. Of the 80 subjects screened, 45 met these criteria. Three patients who were positive by PCR withdrew consent prior to randomization, leaving 42 for randomization. Twenty-six of 80 subjects screened (33%) had undergone a synovial biopsy; of these 26 subjects, 16 (62%) were positive for *Chlamydia* by PCR, and 15 of these were randomized to treatment. Of the 15 subjects randomized based on positive findings on synovial tissue analysis, 5 also had positive findings on PCR of PBMCs. Twenty-seven of 42 subjects (64%) were randomized based on a positive-by-PCR PBMC analysis for *Chlamydia* (no synovial tissue was obtained from these 27 subjects).

Of the 27 patients randomized based on PCR positivity for *Chlamydia* in PBMCs, 17 were receiving active treatment and 10 were receiving placebo (Table 3). Twelve of 17 subjects receiving active treatment became negative by PCR at month 6, and 3 of 10 receiving placebo cleared at month 6 (P = 0.057) (subjects without a blood sample at month 6 were considered positive by PCR). Seven of 15 subjects randomized based on a synovial tissue analysis that was positive by PCR underwent a repeated synovial biopsy in the same joint after 6 months of treatment; 6 of these subjects were randomized to combination antibiotics

Table 3. PCR results*

	Combination antibiotics (n = 27)		Placebo (n = 15)	
	Screening PCR	Month 6 PCR	Screening PCR	Month 6 PCR
PBMCs positive for Ct	12	3	7	5
PBMCs positive for Cpn	3	2	2	1
PBMCs positive for both Ct and Cpn	2	0	1	1
PBMC clearance at month 6	NA	12/17 (71)	NA	3/10 (30)
Synovial tissue positive for Ct	6	2/4	3	0/1
Synovial tissue positive for Cpn	3	0/2	1	ND
Synovial tissue positive for both Ct and Cpn	1	ND	1	ND
Synovial tissue clearance at month 6	NA	4/6 (67)	NA	0/1 (0)

* Values are the number or number (%) of subjects. Of the 27 subjects who received combination antibiotics, 17 were positive for *Chlamydia* in peripheral blood mononuclear cells (PBMCs) by polymerase chain reaction (PCR) and 10 were positive for *Chlamydia* in synovial tissue by PCR. Of the 15 subjects who received placebo, 10 were positive for *Chlamydia* in PBMCs by PCR and 5 were positive for *Chlamydia* in synovial tissue by PCR. Ct = *Chlamydia trachomatis;* Cpn = *Chlamydophila (Chlamydia) pneumoniae;* NA = not applicable; ND = not done.

and 1 to placebo. Four of 6 subjects receiving active treatment were negative by PCR analysis of synovial tissue at month 6; the subject receiving placebo with synovial tissue available at month 6 remained positive by PCR. Combining the PCR data from PBMCs and available synovial tissue, 16 of 23 subjects (70%) receiving combination antibiotics and 3 of 11 subjects (27%) receiving placebo cleared at month 6 ($P = 0.03$).

As stated, 17 responders had received combination antibiotics and 3 responders had received placebo. Two of the 17 responders receiving active treatment who were randomized based on a synovial tissue analysis that was positive by PCR did not undergo a repeat synovial biopsy at month 6, leaving 15 subjects with PCR data at baseline and at month 6. Thirteen of these 15 responders (87%) became negative for *Chlamydia* by PCR at month 6. Of the 3 placebo-treated responders, 1 became negative by PCR at month 6.

PCR analyses of PBMCs were performed again at month 9 (3 months after completion of the study drugs). Regarding the 12 of 17 subjects whose PBMCs became negative by PCR at month 6, PBMCs from all but 2 remained negative by PCR at month 9.

Safety. Table 4 lists the serious AEs and any AEs that occurred more than once in either group. Although both serious AEs occurred in the placebo group, neither was believed to be related to the study drugs (seizures [n = 1] and stabbing [n = 1]). None of the adverse events was statistically more likely to occur in subjects receiving combination antibiotics than in those receiving placebo. The most common AEs in subjects receiving combination antibiotics were gastrointestinal in nature (nausea, diarrhea, abdominal pain, gastroesophageal reflux disease [GERD]). Only 1 subject had to discontinue study medications due to gastrointestinal AEs; this subject was receiving placebo.

Table 4. Adverse events*

	Combination antibiotics (n = 27)	Placebo (n = 15)
Serious adverse events	0 (0)	2 (13)
Any adverse event	22 (81)	10 (67)
Nausea	6 (22)	1 (7)
Abdominal pain	3 (11)	1 (7)
Diarrhea	5 (19)	1 (7)
GERD	2 (7)	0 (0)
Arthralgia	2 (7)	1 (7)
Rash	2 (7)	0 (0)
Viral/upper respiratory infection	3 (11)	1 (7)
Vaginal candidiasis†	2/12 (17)	1/6 (17)

* Values are the number (%) of subjects. There were no significant differences between the groups. GERD = gastroesophageal reflux disease.
† There were 12 women in the combination antibiotics group and 6 women in the placebo group.

DISCUSSION

This randomized 9-month prospective, blinded study demonstrated that compared with placebo, a 6-month course of combination antibiotics resulted in a significantly higher response rate in patients with chronic *Chlamydia*-induced ReA. Although cases of acute ReA often remit spontaneously, the fact that all of the patients in this study had disease duration of at least 6 months (mean >10 years) makes it extremely unlikely that their ReA would improve or resolve spontaneously.

Many of the individual clinical response measurements (e.g., modified SJC, TJC, physician's global assessment of disease activity on a VAS, ESR) also improved significantly in subjects who received active treatment compared with those who received placebo. Twenty-two percent of the subjects receiving combination antibiotics believed that their ReA symptoms had completely resolved. Finally, only those subjects who were positive for *Chlamydia* by PCR were randomized to treatment, and significantly more subjects in the active treatment group than in the placebo group became negative by PCR at month 6.

Importantly, this is the first blinded study to indicate a benefit of prolonged combination antimicrobial therapy in patients with chronic *Chlamydia*-induced ReA. The results of previous trials assessing antimicrobial therapy in the setting of ReA have been equivocal. One previous trial which suggested that antimicrobials are efficacious in the setting of ReA analyzed this treatment approach in acute ReA only, and not in patients with chronic disease (25). Notably, all previous trials evaluated antimicrobial monotherapy (rather than combination therapy), and only one (28) attempted to restrict enrollment to patients with the postchlamydial form of ReA. It seems likely to us that these 2 important changes in the study protocol resulted in improved efficacy. Our data suggest that it is advantageous to attempt attenuation of chlamydial production of HSPs with rifampin, which binds to the β-subunit of prokaryotic RNA polymerase and thereby prevents initiation of transcription of HSPs (29), in combination with antibiotics that block protein synthesis to ensure eradication of the persistent form of this organism. This treatment is best accomplished with a combination of antibiotics for a prolonged period of time, as is the case with other persistent intracellular organisms (i.e., *Mycobacterium tuberculosis* and *Helicobacter pylori*).

Probably the most significant aspect of the present trial is that it suggests an avenue of effective therapy in a patient cohort with chronic arthritis not responsive to NSAIDs or DMARDs. Persistently infecting *Chlamydia* are recognized to be the driving force underlying *Chlamydia*-induced ReA, and organisms in that infectious state have been demonstrated to be refractory to antibiotic treatment (21). The data presented here indicate that while these viable, persistently infectious organisms respond poorly to single antibiotic treatment, they do appear to be susceptible to combination antimicrobial therapy. Thus, a cure theoretically exists. Our study subjects had a mean disease duration of $>$10 years; more than 20% of patients receiving active treatment believed that their disease process did completely resolve, and 26% of these same subjects had a $\geq$70% response by post hoc analysis. This observation is strengthened by the fact that significantly more subjects receiving active treatment became negative by PCR at month 6. Further, there was no indication of clinical worsening from month 6 to month 9, demonstrating that the response was sustained after cessation of antibiotic therapy.

The most common AEs in patients receiving active treatment were gastrointestinal in nature. This is in keeping with the known side effects of the medications studied. Doxycycline is known to cause GERD and esophagitis (43), and azithromycin can cause nausea, abdominal pain, and diarrhea; this has been demonstrated in trials assessing the long-term administration of azithromycin in other disease states (44). These AEs were mild in nature, and no subject receiving active treatment had to discontinue study drugs because of gastrointestinal symptoms.

Some questions remain to be addressed. This study did not determine which combination of antibiotics is the most effective, and it was not powered to compare the 2 different antibiotic regimens. In spite of this limitation, 5 of 6 patients who believed that their disease went into remission were receiving the combination of azithromycin and rifampin, and 33% of those randomized to this treatment strategy achieved remission; these data suggest that this combination might be superior to the other tested.

As stated above, in vitro data have shown that this same combination of antibiotics is capable of eradicating *Chlamydia* in a cell culture model 8 days after infection (22). The most appropriate dosing for long-term administration remains unknown, particularly with azithromycin, and the proper duration of therapy can still be questioned. Of course, there may well be more efficacious alternative antimicrobial combinations. The long-term administration of antibiotics also poses the risk of bacterial resistance. This could be true for the target organism itself or for normal flora. The month 6 PCR data are reassuring that this is not the case for *Chlamydia*, but we have no long-term data from these patients regarding normal flora. Twelve of 17 subjects randomized based on positive results on PCR of PBMCs cleared at month 6; PBMCs from 2 of these 12 subjects again were positive (both for *C trachomatis*) by PCR at month 9. Although the numbers are small, this suggests possible reinfection from a sexual partner who is an asymptomatic carrier of that organism. More data are needed in this regard. Also, the vast majority of patients with ReA have peripheral arthritis, and many have axial involvement. This was true of the patients in our trial,

but 3 subjects (7%) had no peripheral arthritis (only axial arthritis) at screening. The relapsing course of the arthritis in some patients with chronic ReA is a possible explanation, but an alternate diagnosis, such as AS, cannot be excluded in these 3 subjects.

Finally, the utility of diagnostic testing with PCR in similar patients in a clinical setting is still unproven. However, recent data suggest that synovial sample PCR analysis is the method of choice to establish the diagnosis of *Chlamydia*-induced arthritis in patients with ReA and undifferentiated oligoarthritis (45). That same study demonstrated that there is no correlation with *Chlamydia*-specific serologic response. This was also true in our study. Other data consistently show that chlamydial serologic testing is subjective (46); it has also been demonstrated that chlamydial serologies have poor reproducibility (47) and may not be chlamydial species specific (48) and perhaps not even *Chlamydia* specific (49). Because of the trial design, we have no data to address whether combination antibiotic treatment would be effective in patients with undifferentiated SpA who are negative for *Chlamydia* on PCR testing. There is potential for false-positive PCR test results, particularly in patients whose PBMCs are positive for *C pneumoniae* by PCR. In this trial, 7 subjects were randomized (5 to active treatment, 2 to placebo) based on a PCR showing positivity for this respiratory pathogen in PBMCs. Of the 5 patients receiving active treatment, only 1 was a responder. Perhaps the results could be improved upon by restricting treatment to those who are positive for *C trachomatis* by PCR.

The results of this study are encouraging for the management of chronic post-*Chlamydia* ReA. These data suggest that there is potential for eradication of this persistent infection and that improvement in the clinical sequelae that are the result of these infections can be achieved in a substantial number of patients. Clearly, more studies are needed. A diagnostic test that is specific for *Chlamydia*-induced ReA would be important in designing such studies. Further study is warranted to obtain the most efficacious combination of antimicrobial therapy, including dosing and duration, as a potential cure for *Chlamydia*-induced ReA.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Carter had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Carter, Inman, Gerard, Hudson.

Acquisition of data. Carter, Espinoza, Sneed, Ricca, Vasey, Valeriano, Stanich, Oszust, Gerard.

Analysis and interpretation of data. Carter, Espinoza, Inman, Stanich, Hudson.

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