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## Randomized Secondary Prevention Trial of Azithromycin in Patients With Coronary Artery Disease and Serological Evidence for *Chlamydia pneumoniae* Infection

### The Azithromycin in Coronary Artery Disease: Elimination of Myocardial Infection with *Chlamydia* (ACADEMIC) Study

Jeffrey L. Anderson, MD; Joseph B. Muhlestein, MD; John Carlquist, PhD; Ann Allen, BS; Sanjeev Trehan, MD; Cindy Nielson, PharmD; Staci Hall; John Brady, BS; Marlene Egger, PhD; Benjamin Horne, BS; Tobin Lim

**Background**—*Chlamydia pneumoniae* commonly causes respiratory infection, is vasotropic, causes atherosclerosis in animal models, and has been found in human atheromas. Whether it plays a causal role in clinical coronary artery disease (CAD) and is amenable to antibiotic therapy is uncertain.

**Methods and Results**—CAD patients (n=302) who had a seropositive reaction to *C pneumoniae* (IgG titers  $\geq 1:16$ ) were randomized to receive placebo or azithromycin, 500 mg/d for 3 days, then 500 mg/wk for 3 months. Circulating markers of inflammation (C-reactive protein [CRP], interleukin [IL]-1, IL-6, and tumor necrosis factor [TNF]- $\alpha$ ), *C pneumoniae* antibody titers, and cardiovascular events were assessed at 3 and 6 months. Treatment groups were balanced, with age averaging 64 (SD=10) years; 89% of the patients were male. Azithromycin reduced a global rank sum score of the 4 inflammatory markers at 6 (but not 3) months ( $P=0.011$ ) as well as the mean global rank sum change score: 531 (SD=201) for active drug and 587 (SD=190) for placebo ( $P=0.027$ ). Specifically, change-score ranks were significantly lower for CRP ( $P=0.011$ ) and IL-6 ( $P=0.043$ ). Antibody titers were unchanged, and number of clinical cardiovascular events at 6 months did not differ by therapy (9 for active drug, 7 for placebo). Azithromycin decreased infections requiring antibiotics (1 versus 12 at 3 months,  $P=0.002$ ) but caused more mild, primarily gastrointestinal, adverse effects (36 versus 17,  $P=0.003$ ).

**Conclusions**—In CAD patients positive for *C pneumoniae* antibodies, global tests of 4 markers of inflammation improved at 6 months with azithromycin. However, unlike another smaller study, no differences in antibody titers and clinical events were observed. Longer-term and larger studies of antichlamydial therapy are indicated. (*Circulation*. 1999;99:1540-1547.)

**Key Words:** *Chlamydia pneumoniae* ■ antibiotics ■ coronary disease

Atherosclerosis, including coronary heart disease, is highly prevalent in Western society and is the single greatest cause of mortality ( $\approx 33\%$  of annual deaths in the United States).<sup>1</sup> Although many risk factors for atherosclerosis are known (eg, hyperlipidemia, hypertension, smoking, and diabetes), much of attributable risk remains unexplained.<sup>2</sup>

#### See p 1538

Pathologically, atherosclerosis involves injury, inflammation, infiltration, degeneration, and thrombosis.<sup>3-6</sup> A role for both the local inflammatory response in plaque (especially macrophages and T lymphocytes) and systemic inflammation in patients at increased risk for coronary events has been

increasingly recognized and documented.<sup>3-7</sup> The stimulus for this inflammatory response is unknown. The possibility that infectious agents may directly or indirectly trigger the cascade of biological and biochemical reactions leading to inflammation, atherosclerosis, and vascular thrombotic events has been raised recently.<sup>8</sup> With regard to coronary artery disease (CAD), cytomegalovirus and *Chlamydia pneumoniae* have received increasing attention. Whether these or other infectious agents are a cause, a cofactor, or an innocent commensal relative to atherosclerosis is uncertain.<sup>8</sup> However, the possibility that chronic inflammatory/degenerative diseases previously thought to be noninfectious may indeed be infectious and treatable with antibiotics is dramatically ex-

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From the Department of Medicine, Division of Cardiology, University of Utah, LDS Hospital, Salt Lake City, Utah.

Correspondence to J. Brent Muhlestein, MD, or Jeffrey L. Anderson, MD, Coprincipal Investigators, Division of Cardiology, LDS Hospital, 8th Ave & C St, Salt Lake City, UT 84143.

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emphified by the response of peptic ulcer disease to antibiotic therapy directed at *Helicobacter pylori*, now known to play a key etiologic role.<sup>9</sup>

*C pneumoniae* is a recently recognized and characterized human pathogen, distinct from other chlamydial species.<sup>10-12</sup> Epidemiological studies have identified *C pneumoniae* as the third most common etiologic agent of bronchitis and pneumonia.<sup>11</sup> Antibody prevalence studies suggest that >50% of adults have been exposed to it.<sup>12</sup> *C pneumoniae*, an obligate intracellular parasite, is known to readily infect mononuclear phagocytes.<sup>8</sup> Macrophages, which derive from monocytes, are characteristically localized within atherosclerotic plaques.<sup>8</sup>

The link between *C pneumoniae* and atherosclerosis first came from seroepidemiological studies from Finland<sup>13</sup> and subsequently from several additional studies from the same and other groups.<sup>14-17</sup> Although a high prevalence of anti-chlamydial antibodies has consistently been found in CAD patients, its predictive value has varied given the high level of exposure in the general adult population.<sup>18</sup> More compelling has been the finding of bacterial antigen, and less commonly other biological evidence for chlamydia, within atherosclerotic tissues.<sup>19-23</sup> Stimulated by a small initial autopsy study from South Africa,<sup>19</sup> we evaluated plaque from 90 consecutive symptomatic CAD patients undergoing coronary atherectomy; specimens were positive by direct immunofluorescence for chlamydial antigen in 79% compared with only 4% (1) of 24 controls.<sup>21</sup> A high prevalence of antigen detection within coronary or carotid plaque has also been noted by others<sup>19-23</sup>; however, detection of chlamydia by electron microscopy or of chlamydial DNA by polymerase chain reaction has been less consistent,<sup>23,24</sup> and the organism has been difficult to culture.<sup>23</sup> The possibility of a pathogenetic role for chlamydia in CAD has been strengthened by recent animal studies in which intranasal infection of rabbits with *C pneumoniae* was shown to induce or accelerate atherosclerosis.<sup>25,26</sup>

Chlamydia, including *C pneumoniae*, are generally sensitive to antibiotic therapy with macrolides and tetracyclines. Azithromycin, a new macrolide antibiotic, is rapidly absorbed and widely distributed into tissues, where it achieves high and persistent concentrations (tissue half-life  $\approx$ 72 hours). Long-term azithromycin therapy has been well tolerated except for minor, mostly gastrointestinal side effects (eg, nausea and diarrhea).<sup>27</sup> Azithromycin prevented the acceleration of atherosclerosis induced by *C pneumoniae* in rabbits.<sup>26</sup> In a pilot clinical study, Gupta et al<sup>28,29</sup> treated survivors of myocardial infarction (MI) (n=60) with 1 to 2 brief courses of azithromycin or placebo; selected markers of inflammation and anti-chlamydial antibody titers fell over a 3- to 6-month period, and there was an apparent reduction in clinical events. Stimulated by these initial observations, we undertook a larger prospective, randomized, secondary prevention study.

## Methods

### Study Design and Objectives

The study was designed as a randomized, double-blind, secondary prevention trial of azithromycin (3-month course) versus placebo in patients with documented CAD to test both laboratory and clinical objectives.

The laboratory hypothesis was that azithromycin therapy, compared with placebo, would reduce a global measure of systemic levels of a cluster of inflammatory markers (C-reactive protein [CRP], interleukin [IL]-1, IL-6, and tumor necrosis factor [TNF]- $\alpha$ ) at 3 months (end of therapy) and 6 months. The secondary laboratory hypothesis was that antibiotic therapy would reduce anti-chlamydial IgG and IgA antibody levels at 6 months.

The clinical hypothesis was that at 6 months (secondary end point, this article) and 2 years (primary end point), cardiovascular events would be reduced in the azithromycin group compared with the placebo group. End-point events were defined as cardiovascular death, resuscitated cardiac arrest, nonfatal MI or stroke, unstable angina requiring hospitalization, and unplanned coronary interventions (catheter based or surgical).

Other clinical objectives included comparison, by treatment group, of all deaths, other cardiovascular hospitalizations or procedures, adverse experiences and drug discontinuations, and clinical infections. Other laboratory comparisons of interest included total leukocyte count and blood pressure and heart rate measurements by group (at 3 and 6 months).

### Patient Qualification, Enrollment, and Randomization

Patients qualified for the study if they had CAD (documented by a previous MI, bypass surgery, or >50% angiographic stenosis of  $\geq$ 1 major coronary artery), were >18 years old, had a life expectancy of >2 years, and gave written informed consent. Exclusion criteria included the following: female capable of child bearing without adequate birth control; New York Heart Association functional class III or IV or left ventricular ejection fraction <25%; MI within 5 days, bypass surgery within 4 weeks, or coronary intervention (any technique) within 3 months; planned CABG or coronary intervention; significant comorbid illnesses, including active malignancy, ongoing drug or alcohol abuse, renal failure requiring dialysis, and liver failure, with a projected life expectancy of <2 years; known intolerance to azithromycin; and chronic macrolide (eg, erythromycin) or tetracycline use.

Patients who met all study criteria and gave informed consent were screened for *C pneumoniae* serum antibody titers to provide  $\approx$ 300 patients for enrollment with anti-chlamydial IgG titers of  $\geq$ 1:16 by microimmunofluorescence (MIF). Enrolled patients were randomized 1:1 to the 2 treatment groups (azithromycin or placebo) by an unblinded, independent party (pharmacist) who was uninvolved in clinical management except for provision of blinded drug supplies; the pharmacist opened an envelope containing the drug assignment. The order of randomization followed a permuted block design (alternating blocks of 4 and 6).

### Supplies and Dosing of Drug and Placebo

Azithromycin, as 250-mg red capsules (Pfizer Laboratories), was purchased from LDS Hospital Pharmacy. Matching placebo capsules were purchased from Jolley's Corner Pharmacy in Salt Lake City, Utah. Enrolled patients were to initiate therapy with 2 capsules (500 mg/d for 3 days) then maintain therapy with 2 capsules (500 mg/wk, taken on Mondays) for 3 months, to be taken  $\geq$ 1 hour before or 2 hours after food.

### Study Flow

Eligibility was determined during a screening visit. Venous blood was drawn from eligible, consenting patients and was tested by MIF for *C pneumoniae* antibodies. Patients with titers  $\geq$ 1:16 were seen  $\approx$ 1 week later for randomization; baseline history and physical examination were completed, blood was drawn, a 12-lead ECG was obtained, and study drug was dispensed. At 3 and 6 months, patients returned to review clinical status and drug compliance; blood was sampled and an ECG obtained. One- and 2-year clinical follow-up was scheduled.

## Laboratory Testing

Screening for anti-*C pneumoniae* antibody (IgG) used a standard MIF test (MRL Diagnostics) on 1:16 diluted serum. Quantitative IgG and IgA measurements used a sensitive, specific ELISA (Savyon Diagnostics Ltd). Tests for CRP (Johnson & Johnson Clinical Diagnostics, Inc), IL-1, IL-6, and TNF- $\alpha$  (Quantikine HS, R&D Systems) were also performed by commercially available methods. Leukocyte counts were determined by the hospital laboratory by standard methods. Tests were run in batches that included sets of baseline and follow-up samples for each patient.

## Statistical Considerations

The study was planned to have 90% power to detect differences in the primary laboratory end points at 3 to 6 months between treatment groups of 0.385 SD for the markers of inflammation, based on estimates from literature studies (eg, References 28 and 29) and assuming a 5% dropout rate. This was satisfied with  $\approx$ 300 patients (150 in each treatment group).

Clinical event rate estimates were based on the pilot study of Gupta et al,<sup>28,29</sup> who reported a reduction in events from 28% to 8% by 6 to 18 months in treated patients who had falling antibody titers versus controls (ie, patients taking placebo or untreated patients with persistently elevated titers). Assuming a placebo event rate of 28%, 150 patients per group provide  $\geq$ 90% power to detect a study drug-induced reduction in event rate to 14.5% at 6 months (secondary clinical end point) and at 2 years (primary clinical end point).

The primary (null) laboratory hypothesis was that there would be no difference between the azithromycin and placebo groups in global change scores from baseline in markers of inflammation measured at 3 and 6 months; the alternate hypothesis was that changes would favor antibiotic therapy. Differences between baseline and 3 and 6 months for individual markers were checked for normality by the Kolmogorov-Smirnov test. Given the expectation of improvement in most or all inflammatory markers and nonnormal distribution of data, the O'Brien nonparametric rank sum test was chosen as the global test of the multiple end points (changes in the 4 inflammatory markers for our primary laboratory analysis).<sup>30,31</sup> A global comparison between the 2 treatment groups was also performed on actual marker values at 3 and 6 months with the O'Brien test used for a secondary analysis. Between-treatment-group comparisons were then made for each marker separately (Mann-Whitney test) to assess which markers contributed to the global result. Otherwise, categorical variables in the 2 groups were compared by  $\chi^2$  testing, and continuous variables were compared by Student's *t* test or Mann-Whitney testing, as appropriate. Assessment of clinical events used the intention-to-treat approach. Laboratory marker analyses excluded only patients without a blood test result.

## Results

### Baseline Patient Characteristics

A total of 430 patients were screened; 333 (77%) had positive *C pneumoniae* serology, and 302 patients consented and were enrolled in the study, with 150 randomly assigned to receive azithromycin and 152 to receive placebo. Overall, patients were  $64 \pm 10$  years of age (mean  $\pm$  SD; range, 30 to 88 years); 89% were male; 60% had suffered a previous MI; 46% had undergone bypass surgery; and 46% had undergone percutaneous coronary interventions. Patient characteristics on admission were well balanced in the randomly assigned treatment groups (Table 1).

Baseline levels of inflammatory markers and antibody titers are shown in Table 2. Individual markers showed substantial interpatient variability, but no significant differences were observed between the 2 treatment groups.

**TABLE 1. Baseline Patient Characteristics**

Characteristic	Azithromycin Group (n=150)	Placebo Group (n=152)	P
<b>Atherosclerotic risk factors</b>			
Age, y (mean $\pm$ SD)	64 $\pm$ 10	63 $\pm$ 11	0.60
Sex, % male	86	91	0.19
Hypertension, %	39	45	0.34
Diabetes, %	11	11	0.97
Smoking, %	38	36	0.85
Family history of CAD, %	69	73	0.43
<b>Indications for inclusion, %</b>			
History of MI	63	58	0.33
History of bypass surgery	65	61	0.45
History of PTCA/stent	42	49	0.20

### Effect of Therapy on Global Inflammatory Score

Overall, measured values of the 4 inflammatory markers were similar at 3 months but were reduced at 6 months in the azithromycin compared with the placebo group: average rank sum scores were 583 (SD=182) versus 593 (SD=168) ( $P=0.64$ ) at 3 months and 532 (SD=203) versus 598 (SD=205) ( $P=0.011$ ) at 6 months in the active and placebo groups, respectively (Figure 1).

Similarly, for the primary study variable of global change score, average rank sum score was similar in the 2 treatment groups for the change in measured values from baseline at 3 months (mean rank sum score, 584 [SD=198] versus 586 [SD=189];  $P=0.92$ ), but it was reduced compared with baseline at 6 months (mean rank sum score, 531 [SD=201] versus 587 [SD=190];  $P=0.027$ ) in the active-drug and placebo groups, respectively (Figure 2).

### Response of Individual Markers of Inflammation

Over the entire study population, IL-1, IL-6, and TNF fell from baseline at 3 months (all  $P < 0.01$ , Wilcoxon rank sum test), then recovered to less than (IL-6), similar to (TNF), or

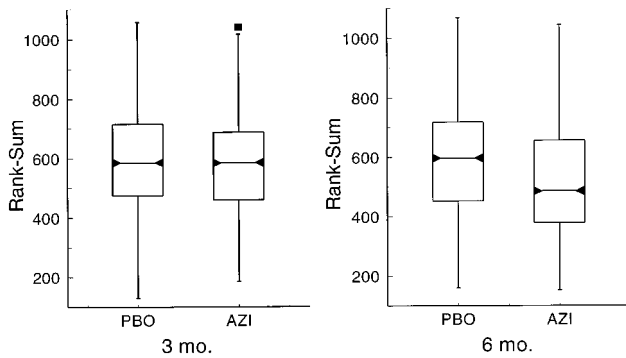
**TABLE 2. Baseline Levels of Inflammatory Markers and Anti-Chlamydial Antibody Concentrations**

Marker	Azithromycin Group	Placebo Group	P*
CRP, mg/dL	0.88 $\pm$ 0.66 (0.70)	0.87 $\pm$ 0.60 (0.60)	0.23
n	146	143	
IL-1, pg/mL	2.87 $\pm$ 2.82 (1.49)	3.03 $\pm$ 2.93 (1.67)	0.51
n	150	149	
IL-6, pg/mL	3.86 $\pm$ 3.06 (2.84)	3.61 $\pm$ 2.87 (2.57)	0.55
n	150	149	
TNF, pg/mL	8.61 $\pm$ 9.20 (4.06)	9.19 $\pm$ 9.20 (5.02)	0.29
n	149	149	
IgG, rvu	2.82 $\pm$ 1.17 (2.95)	2.87 $\pm$ 1.22 (2.79)	0.66
n	110	108	
IgA, rvu	2.84 $\pm$ 1.56 (2.66)	2.94 $\pm$ 1.62 (2.82)	0.77
n	139	134	

rvu indicates relative value units.

\*Mann-Whitney.

Plus/minus values are mean  $\pm$  SD, with median values in parentheses.



**Figure 1.** Comparison of global rank sum scores (O'Brien non-parametric test) for actual values of variables by treatment group at 3 months ( $P=0.64$ ; left) and 6 months ( $P=0.011$ ; right). Boxes encompass 25th to 75th percentile rank sums; horizontal lines within boxes represent median; thin vertical lines beyond boxes define 5% and 95% percentiles; outliers are given by individual black squares. PBO indicates placebo; AZI, azithromycin.

greater than baseline (IL-1) at 6 months. The effect of treatment on individual inflammatory markers is shown in Tables 3 and 4 and Figure 3; all markers tended to show lower values (ranks) at 6 months with azithromycin, although the magnitude and significance of the responses varied.

### C-Reactive Protein

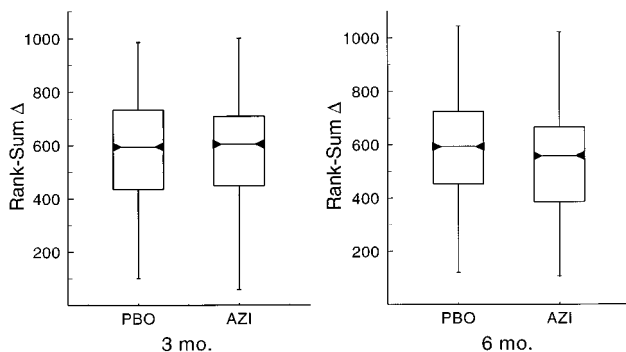
CRP measurements were variable in the study population, with most falling in the normal range. However, a significant difference by treatment group in the change score was noted at 6 months but not 3 months (Table 4; Figure 3), with a mean rank of  $\Delta$ CRP values of 128 in azithromycin versus 150 in placebo patients ( $P=0.017$ ).

### Interleukin-1

Therapy affected IL-1 measurements at 6 but not 3 months, preventing the rebound to above-baseline values seen in placebo patients (Table 3). Median IL-1 at 6 months was 1.45 pg/mL in patients taking active drug versus 2.20 pg/mL in patients taking placebo ( $P=0.027$ ). Change scores did not achieve significance, however (Table 4).

### Interleukin-6

IL-6 levels fell in both treatment groups at 3 months (Table 3) compared with baseline (Table 2), but only active therapy



**Figure 2.** Comparison by treatment of global rank sum change scores (O'Brien nonparametric test) for primary study variable: change from baseline at 3 months ( $P=0.92$ ; left) and 6 months ( $P=0.027$ ; right). Format and abbreviations otherwise as in Figure 1.

**TABLE 3. Values of Inflammatory Markers at 3 and 6 Months by Treatment**

Marker		Mean (SD)	Median	$P^*$
<b>3 Months</b>				
CRP, mg/dL				
Placebo	143	0.87 (0.49)	0.70	
Azithromycin	146	0.92 (0.84)	0.60	0.77
IL-1, pg/mL				
Placebo	146	2.09 (2.25)	1.26	
Azithromycin	149	2.08 (2.47)	1.04	0.43
IL-6, pg/mL				
Placebo	146	3.27 (2.43)	2.45	
Azithromycin	149	3.18 (2.57)	2.40	0.84
TNF, pg/mL				
Placebo	146	6.27 (8.12)	3.09	
Azithromycin	149	6.29 (7.73)	3.30	0.85
<b>6 Months</b>				
CRP, mg/dL				
Placebo	144	1.00 (1.27)	0.60	
Azithromycin	143	0.87 (1.02)	0.60	0.14
IL-1, pg/mL				
Placebo	145	2.72 (2.27)	2.20	
Azithromycin	145	2.24 (1.97)	1.45	0.027
IL-6, pg/mL				
Placebo	128	5.20 (6.99)	2.72	
Azithromycin	128	4.30 (6.38)	2.42	0.17
TNF, pg/mL				
Placebo	143	6.31 (6.36)	4.66	
Azithromycin	144	5.94 (6.13)	4.33	0.25

\*Mann-Whitney test between treatment groups.

prevented a subsequent increase back to baseline values (Tables 3 and 4; Figure 3). At 6 months, mean rank for  $\Delta$ IL-6 was 118 in active-therapy versus 139 in placebo-treated patients ( $P=0.023$ ).

### Tumor Necrosis Factor

TNF levels tended to decrease similarly in both groups at 3 months and return only partially toward baseline by 6 months. The slight trend to lower TNF values at 6 months with active therapy did not approach significance ( $P=0.25$ ; Table 3).

### Effect of Therapy on Anti-Chlamydial Antibody Titers

Table 4 shows quantitative measures of anti-*C pneumoniae* IgG and IgA titers at baseline and 3 and 6 months in the 2 treatment groups. Titers were stable or tended to increase slightly over time. No differences in changes in titers of either immunoglobulin by treatment group were observed at either 3 or 6 months.

### Effect of Therapy on Early (<6 Months) Clinical Events

Table 6 shows the composite of clinical cardiovascular events that occurred within 6 months in the 2 treatment groups (our

**TABLE 4. Inflammatory Marker Change Scores at 3 and 6 Months by Treatment Group**

Marker	Mean (SD)	Median (5th and 95th Percentiles)	<i>P</i> *
<b>3 Months</b>			
$\Delta$ CRP, mg/dL			
Placebo	143 0.001 (0.44)	0.0 (−0.5, 0.6)	0.67
Azithromycin	144 0.036 (1.03)	0.0 (−0.7, 0.5)	
$\Delta$ IL-1, pg/mL			
Placebo	146 −0.97 (3.45)	−0.34 (−7.04, 5.24)	0.76
Azithromycin	149 −0.77 (3.33)	−0.32 (−7.14, 5.67)	
$\Delta$ IL-6, pg/mL			
Placebo	146 −0.37 (2.95)	−0.02 (−6.42, 3.38)	0.19
Azithromycin	149 −0.69 (3.21)	−0.37 (−6.86, 4.27)	
$\Delta$ TNF, pg/mL			
Placebo	146 −2.98 (12.05)	−1.02 (−24.84, 18.07)	0.34
Azithromycin	149 −2.44 (12.54)	−0.30 (−23.48, 18.05)	
<b>6 Months</b>			
$\Delta$ CRP, mg/dL			
Placebo	138 0.12 (1.22)	0.0 (−1.0, 1.4)	0.017
Azithromycin	139 −0.001 (1.15)	0.0 (−0.7, 0.6)	
$\Delta$ IL-1, pg/mL			
Placebo	145 −0.32 (3.59)	0.09 (−6.73, 5.12)	0.29
Azithromycin	145 −0.60 (3.12)	−0.19 (−6.70, 4.19)	
$\Delta$ IL-6, pg/mL			
Placebo	128 1.80 (7.22)	0.28 (−6.16, 20.00)	0.023
Azithromycin	128 0.55 (6.87)	−0.28 (−7.76, 13.05)	
$\Delta$ TNF, pg/mL			
Placebo	143 −2.76 (10.91)	1.07 (−20.77, 14.80)	0.66
Azithromycin	143 −3.04 (10.80)	0.69 (−22.14, 7.68)	

$\Delta$  indicates change (difference) from baseline (ie, 3- or 6-month value minus baseline value).

\*Mann-Whitney test for between-group comparison.

secondary time point; primary end point at 2 years). A total of 9 cardiovascular events occurred in the azithromycin group, and similarly, 7 events occurred in the placebo group. The distribution of events was also generally similar in the 2 groups.

### Effect of Therapy on Total Leukocyte Count and Vital Signs

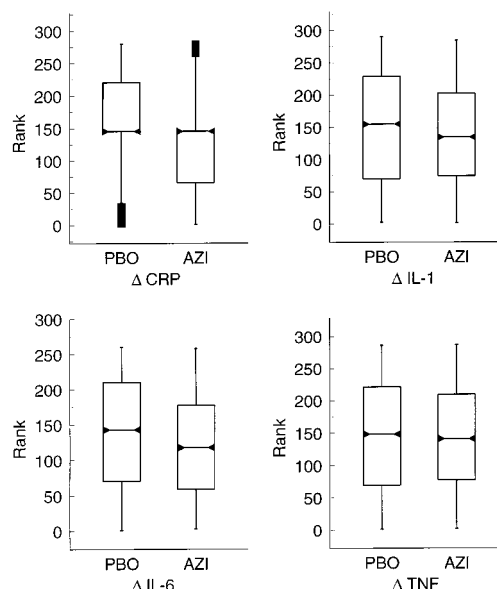
Leukocyte counts did not change significantly over time in either group. Vital signs were unchanged over 6 months, with no significant differences emerging between groups in heart rate or systolic or diastolic blood pressure.

### Effect of Therapy on Rates of Infection

Azithromycin reduced the number of clinical infections over the 3-month treatment period (12 versus 27;  $P=0.011$ ), including those that required antibiotics (1 versus 12;  $P=0.002$ ). Two patients in the active-therapy group developed a secondary yeast (candidal) infection.

### Drug Tolerance and Compliance

Compliance was good to excellent; 89% of active-therapy and 85% of placebo-treated patients followed the treatment



**Figure 3.** Ranks of change scores of 4 individual inflammatory markers at 6 months compared with baseline in the 2 treatment groups.  $\Delta$ CRP,  $\Delta$ IL-1,  $\Delta$ IL-6,  $\Delta$ TNF indicate change in CRP, IL-1, IL-6, and TNF, respectively, at 6 months for placebo (PBO) and azithromycin (AZI) groups. Format as in Figure 1.

plan without deviation. Only 2 active-therapy and 3 placebo-treated patients permanently discontinued therapy  $>2$  weeks early. Overall, 36 (24%) of patients treated with azithromycin and 17 (11%) of patients taking placebo experienced some adverse effect during the 3-month treatment period ( $P=0.003$ ). Adverse effects were believed to be possibly or probably drug related in 25 of active and 11 placebo-treated patients ( $P=0.011$ ). They were usually mild, occasionally moderate, and most frequently gastrointestinal (31 of the active-therapy patients, 12 of the placebo-treated patients;  $P=0.002$ ), including nausea, diarrhea, or flatulence. A yeast infection was observed in 2 patients taking active therapy; 1 case of esophageal candidiasis was moderately severe, requiring drug discontinuation and specific therapy.

## Discussion

### Summary of Study Results

This double-blind, randomized, moderately large, secondary prevention study in CAD patients with serological evidence

**TABLE 5. Effect of Therapy on Anti-Chlamydial Antibody Levels**

Measure (n)	Azithromycin	Placebo	<i>P</i> *
Change score (rvu), 3 mo vs baseline			
$\Delta$ IgG (146)	1.11 $\pm$ 1.87 (0.89)	1.25 $\pm$ 2.08 (0.79)	0.90
$\Delta$ IgA (87)	0.76 $\pm$ 1.09 (0.85)	1.14 $\pm$ 0.92 (1.15)	0.093
Change score (rvu), 6 mo vs baseline			
$\Delta$ IgG (216)	0.41 $\pm$ 2.12 (0)	0.30 $\pm$ 1.84 (−0.05)	0.39
$\Delta$ IgA (271)	0.27 $\pm$ 0.86 (0.16)	0.27 $\pm$ 0.84 (0.11)	0.68

rvu indicates relative value units.

\*Mann-Whitney.

**TABLE 6. Effect of Therapy on Early (6 Month) Clinical Events**

Event	Azithromycin	Placebo	P
Composite CV end point	9	7	0.60
CV death	1	2	
Nonfatal MI	2	1	
Hospitalization for unstable angina	4	2	
Unplanned revascularization	3	4	
Nonfatal stroke	1	1	

CV indicates cardiovascular.

of prior *C pneumoniae* exposure showed no difference in global inflammatory marker scores at the end of the 3-month treatment period, but at 6 months, the global difference score was lower in the active-therapy group, both for change scores ( $P=0.03$ ) and actual value scores ( $P=0.01$ ). All measures trended in a favorable direction, with significance reached for CRP, IL-1, and IL-6 on one or the other test. Unlike in a previous report,<sup>28</sup> antibody titers were unaffected by therapy. Also in contrast to 2 smaller studies,<sup>28,29,32</sup> no difference emerged by 6 months in clinical events. The results of these earlier, smaller studies thus appear overly enthusiastic; however, we believe our results should encourage rather than discourage additional clinical and laboratory studies based on more realistic expectations. Testing of similar and additional inflammatory markers should be undertaken, including others that assess monocyte/macrophage activation (eg, tissue factor and CD11b) and endothelial activation (eg, soluble intercellular adhesion molecule and soluble vascular cell adhesion molecule). Larger and longer studies of clinical outcomes should also proceed.

The utility of our randomized control design is emphasized by the fall in levels of 3 inflammatory markers at 3 months, even in the placebo group; the cause of this unexpected placebo effect is uncertain but might involve, among other things, increased patient compliance with aspirin on study entry or a seasonal effect on inflammation.

### Pathophysiological Considerations

How could infectious agents promote atherogenesis? Libby et al<sup>8</sup> suggest that both direct and indirect effects on vascular wall cells may be involved: infection may cause lethal lytic damage, or infected cells may survive but show altered function. Endothelial dysfunction may include increased procoagulant activity, reduced intrinsic fibrinolysis, increased leukocyte adhesion, and increased cytokine production. Smooth muscle cell dysfunction may be associated with increased proliferation, reduced apoptosis, increased cholesterol esterification, and increased cytokine production. Recruitment and activation of leukocytes also result. Indirect effects on vascular cells may accompany infection or activation of vessel-associated leukocytes; mononuclear phagocytes may show increased procoagulant and reduced fibrinolytic behavior, altered lipid metabolism, increased cytokine production, and release of toxic oxygen species. T cells may show increased proliferation, cytokine production, and lytic activity for vascular wall cells.

With respect to *C pneumoniae*, little is currently known regarding the biology of vascular wall infection.<sup>8,33</sup> *C pneumoniae* commonly infects mononuclear phagocytes, and chlamydial species can infect epithelial cells persistently under certain conditions. It is known that the chlamydial life cycle involves the elementary body (extracellular, infectious form) and the reticulate body (intracellular, replicative form); under conditions of stress, a metabolically inactive but viable form, the persistent body, has also been described.<sup>34</sup> Thus, chlamydia are good candidates to cause chronic, persistent, nonlytic cellular infection.

It is known that infection increases the level of production of the inflammatory cytokines IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ; TNF and IL-1 upregulate IL-8.<sup>35</sup> Hepatocytes respond to IL-1 and IL-6 by producing CRP,<sup>36</sup> and CRP can induce expression of monocyte tissue factor.<sup>37</sup> Thus, these interrelated inflammatory mediators, all resulting from and/or enhancing macrophage/monocyte activation, were used together in the present study to define a global marker of inflammation/macrophage activation. The use of these multiple, interdependent markers was more attractive than 1 arbitrarily selected variable. Indeed, the findings of our study suggest an overall beneficial action of antibiotic therapy on serum markers of inflammation, although the effect was somewhat delayed (to 6 months) and modest in extent.

### Comparisons With Previous Antibiotic Trials

Our performance of a secondary prevention antibiotic study was stimulated by the finding of a surprisingly high prevalence of chlamydial antigen in coronary atheroma extracted by us at atherectomy, together with promising results from antibiotic therapy in our animal model and in a pilot clinical study from the United Kingdom.<sup>26,28,29</sup> In one approach to examining the question of whether chlamydia play a pathogenic or commensal role, we repeatedly infected 30 rabbits intranasally with *C pneumoniae* or saline, then randomly assigned them to 2 months of azithromycin or no therapy and assessed aortic maximal intimal thickness (MIT) and plaque area index (PAI) at 3 months.<sup>26</sup> MIT and PAI were increased 3-fold in infected, untreated animals compared with uninfected controls; in contrast, infected animals treated with antibiotics showed MITs and PAIs similar to uninfected animals. These results suggested that *C pneumoniae* infection may accelerate atherogenesis and antichlamydial therapy may prevent it, at least in some models.

Gupta et al<sup>28,29</sup> stimulated interest in antibiotic trials in humans with a recent pilot study in the United Kingdom: 60 survivors of acute MI with persistently elevated anti-chlamydial antibody titers ( $\geq 1:64$ ) were randomized to receive placebo, a single 3-day course of azithromycin (500 mg/d), or 2 courses 3 months apart. At 6 months, anti-chlamydial antibody titers fell to  $< 1:16$  in 43% of patients in the azithromycin group versus 10% of subjects in the placebo group ( $P=0.02$ ). Reductions in some of several measured markers of inflammation were also observed, specifically, monocyte/macrophage tissue factor and the surface adhesion molecule CD11b.<sup>28</sup> In addition, compared with patients in the placebo group plus a nonrandomized group with high anti-

body titers, azithromycin-treated patients showed an apparent reduction in cardiovascular events within 6 to 18 months (from 28% to 8%;  $P=0.03$ ).

Subsequent to the initiation of our study, Gurfinkel et al<sup>37</sup> reported on a pilot antibiotic trial from Argentina: 202 patients presenting with unstable angina or non-Q-wave MI were randomized on hospital admission to roxithromycin, 150 mg twice daily, or placebo for 30 days. Rates of recurrent angina (2 versus 5), acute MI (0 versus 2), death (0 versus 2), or any event (2 versus 9) tended to be reduced at 1 month (adjusted  $P=0.064$ ). The study suffers from the small number of events, poor characterization of patients, and short follow-up but raises the question of the role of inflammation in unstable coronary syndromes and the possibility that antibiotic therapy might provide benefit in the acute or subacute setting.

Our study results contrast with these earlier results in several ways. We randomized a much larger sample than Gupta (300 versus 60 patients) and gave more intensive antibiotic therapy (7.5 versus 1.5 to 3.0 g of azithromycin over 3 months) but did not confirm *C pneumoniae* antibody titer responsiveness. Furthermore, we did not confirm a dramatic early reduction in clinical events, although a comparison at 6 months cannot be strictly made between studies because the time distribution of observed events was not reported in their published report.<sup>29</sup> The study of Gurfinkel et al<sup>32</sup> was of intermediate size (202 patients); their report of a strong trend in reduction in number of adverse events (2 versus 9) at 1 month also stands in contrast to our study of a much larger patient follow-up (1800 versus 202 patient-months). Because Gurfinkel et al<sup>32</sup> studied acute coronary syndrome patients, differences in outcome based on differences in the patient population, although unlikely, cannot be entirely excluded. In keeping with our study, they found no reduction in anti-chlamydial antibody titers in response to therapy, at least at 1 month.

### Study Strengths and Limitations

This study adds substantially to the small amount of previous work relating *C pneumoniae* to atherosclerosis in humans<sup>8</sup> and antibiotic therapy directed at chlamydia in patients with CAD manifestations.<sup>29,32</sup> The validity of the statistical inferences of the study are bolstered by its prospective, randomized, double-blind design and moderately large size. The power of the study is excellent for the laboratory end points. Indeed, overall stabilization of marker levels was observed. For the clinical end point, it was recognized that event rates at 6 months might be too low to demonstrate a clear therapeutic benefit, although previous pilot studies suggested this possibility.<sup>29,32</sup> Hence, the primary clinical end point is at 2 years. Given our event-rate comparison at 6 months, however, we believe that even larger studies of at least a few thousand patients and at least a few years' follow-up should be pursued.

The optimal dose and duration of azithromycin therapy are unknown in the setting of chronic CAD. We based our 3-month course on the conjecture that a persistent, low-grade, indolent infection might require a longer duration of therapy than acute infections. Also, therapy in the promising pilot

study of Gupta et al extended over 3 months, although our treatment course was much more intense. Safety assessment for the use of chronic azithromycin is not yet available beyond 3 to 6 months. Chlamydia in a persistent, metabolically inactive state might not be immediately susceptible to antibiotic therapy. Longer-term and/or intermittent (eg, monthly) treatment may be required to ensure lasting benefit. Also, reinfection with *C pneumoniae* is known to be possible, even in adulthood, and appears to occur on the order of 2% to 3% annually.

An additional limitation is the uncertainty that the effects of azithromycin are due to a specific antichlamydial action and not a more general anti-infectious or anti-inflammatory effect. A similar uncertainty arises in attempting to explain the recently demonstrated benefits of tetracycline therapy in rheumatoid arthritis.<sup>38</sup> Although additional work will be needed to resolve this concern, a beneficial effect on atherosclerosis by either specific or nonspecific mechanisms is of interest.

We included only seropositive patients in this study; inclusion of seronegative patients in future studies might help to answer the question of specific versus nonspecific antichlamydial effects of azithromycin. However, seropositivity is an imperfect marker of persistent chlamydial arteritis and is associated with uncertain, possibly substantial rates of false positivity and negativity. In a small subgroup of patients with atherectomy samples positive for chlamydial antigen, 8 of 9 also were seropositive (J.B. Muhlestein, MD, et al, unpublished observations, 1997). Chronic vascular infection with chlamydia, not prior systemic exposure (eg, acute respiratory infection), underlies our study hypothesis, but a direct means of detection of chronic vasculitis, short of atherectomy, is not yet available.

### Conclusions

We tested the hypothesis that *C pneumoniae* infection may be an additional atherosclerotic risk factor in patients with CAD by performing a prospective, double-blind, randomized, secondary prevention trial ( $n=302$  patients) with the potent antichlamydial antibiotic azithromycin. We found that in CAD patients positive for *C pneumoniae* antibodies, a global test on a cluster of 4 inflammatory markers showed lower rank scores with active therapy at 6 but not 3 months, both for change scores ( $P=0.03$ ) and for actual value scores ( $P=0.01$ ). However, no differences were observed in antibody levels or clinical events at 6 months. Although our results suggest that 2 previous smaller studies were overly enthusiastic in their conclusions, our findings should stimulate rather than discourage larger and longer-term clinical trials, as well as preclinical research, to explore the intriguing but as yet unproved hypothesis that infectious agents may play a role as causative factors or cofactors in at least a subgroup of patients with CAD who might be amenable to antibiotic or other preventive therapies.

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