Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Atherosclerosis is increasingly viewed as an inflammatory process. A number of infectious agents have been implicated in the pathogenesis of coronary artery disease. *Chlamydia pneumoniae* has been the most popular and well-studied of these pathogens. It is difficult to prove a causal relationship which requires the fulfillment of Koch’s postulates, first developed in the late 1800s, to establish an infectious agent as the cause of a disease process. This paper reviews the evidence for and against *Chlamydia pneumoniae* infection as a contributing factor to atherosclerosis disease. It examines seroepidemiologic and histopathologic studies as well as animal models using Koch’s postulates and then provides an analysis of current clinical trial data.

Infectious agents have been proposed as a potential cause of atherosclerosis since the late 1800s and early 1900s (Table 1). In a paper published in 1911, Frothingham stated, “Acute infectious diseases may cause some pretty general lesion throughout the arterial system, either from the diffuse action of toxins or from a widespread invasion of the arterial system by the infecting organisms. The exact nature of these lesions in human cases and their final result have not been so well worked out.” Nearly 100 years later, we are still grappling with the same question. This certainly is not the first instance of an infection being linked to a chronic disease. Well-documented associations that have revolutionized disease management include *Helicobacter pylori* and peptic ulcer disease, human papillomavirus and cervical cancer, human herpesvirus 8 and Kaposi sarcoma, and hepatitis B and hepatocellular carcinoma. Although diseases such as scarlet fever, diphtheria, and measles were cited as plausible contributors to the atherosclerotic process in the early 20th century, the control of these pathogens over the ensuing years has given rise to a new cast of suspect agents.

**Infectious Agents Linked to Atherosclerosis in Recent Literature**

Infectious agents implicated in atherosclerosis over the past 10 to 15 years include cytomegalovirus (CMV), herpes simplex virus (HSV), *H pylori*, and *Chlamydia pneumoniae*. Like the pathogens formerly under question, exposure to these organisms occurs relatively early on in life, setting the stage for their potential role in the initiation and pathogenesis of the atherosclerotic process. Furthermore, these organisms are similar in that they are all obligate intracellular pathogens capable of maintaining a chronic persistent state in the host.

Data linking herpes viruses to atherosclerosis first came from Marek avian herpes virus, where infection of disease-free chickens with the virus produced arterial lesion. A member of the Herpesviridae family, CMV has been associated with an increased risk of atherosclerosis and death from coronary disease after cardiac transplant. Prior CMV infection has also been linked to increased risk of restenosis after percutaneous coronary intervention. However,
data are conflicting with several prospective, nested, case-control studies showing disparate results. Whereas the Atherosclerosis Risk in Communities Study found that high levels of CMV antibodies correlated with an increased risk of coronary artery disease (CAD) during a 5-year follow-up, the Physicians Health Study did not find any association between CMV or HSV antibodies and myocardial infarction (MI) or stroke over a 12-year follow-up period. The Cardiovascular Health Study found that antibodies to HSV but not CMV were associated with increased risk of MI and cardiovascular death in elderly patients.

A number of seroepidemiological studies have suggested an association between \( \text{H} \text{ pylori} \) and coronary disease, but many of these studies did not adjust appropriately for potential confounders such as socioeconomic status. However, 1 case-control study which did adjust for age, sex, other cardiac risk factors, and social class found a correlation between \( \text{H} \text{ pylori} \) and coronary disease; this relationship may have been caused by an increased prevalence of more virulent \( \text{H} \text{ pylori} \) strains in these patients. Unlike the other organisms in question, \( \text{H} \text{ pylori} \) has not been detected in atherosclerotic plaque.

In addition, several studies have shown that infectious burden, for example, the number of pathogens to which an individual has been exposed as determined by serology, is associated with the extent of atherosclerotic disease and risk for cardiovascular death. The presence of chronic respiratory tract, urinary tract, dental, or other infection was associated in 1 study with a 4-fold increase in the risk of carotid atherosclerosis.

Conflicting data in the literature regarding the association between infectious pathogens and coronary events make it difficult to draw definitive conclusions regarding their role in disease pathogenesis and therefore potential therapeutic interventions. \( \text{C} \text{ pneumoniae} \) has been the best described of these pathogens, and this paper will thus review the current data on \( \text{C} \text{ pneumoniae} \) and its potential role in the pathogenesis of atherosclerotic disease.

### Microbiology and Epidemiology of \( \text{C} \text{ pneumoniae} \)

\( \text{C} \text{ pneumoniae} \) is an obligate intracellular bacterium capable of chronic or persistent infection. It was first identified 20 years ago as a cause of acute upper and lower respiratory tract infections. It has a unique developmental cycle involving 2 morphological forms, the elementary body, and the reticulate body. The former is the infectious form and adapted to extracellular survival, whereas the latter is the metabolically active and dividing form, adapted for intracellular multiplication. In addition, the organism may evolve into a “persistent body,” an intracellular, metabolically inactive, nonreplicating but viable form that allows it to maintain chronic infection and thereby a state of chronic inflammation that may contribute to the atherosclerotic process.

\( \text{C} \text{ pneumoniae} \) infection is ubiquitous, with an antibody prevalence of 50% by age 20 and 70% to 80% at age 60 to 70. It accounts for 10% of community-acquired pneumonia and 5% of pharyngitis, bronchitis, and sinusitis.

### Koch’s Postulates

In 1886, Robert Koch developed the following criteria that must be satisfied to prove that a specific organism causes a specific disease:

1. The pathogen must be present in nearly all cases of disease.
2. The pathogen must be isolated from the diseased host and grown in culture.
3. The disease must be reproduced when the culture is inoculated into the healthy host.
4. The organism must be recovered from the experimentally infected host.

Koch used these criteria to establish bacillus anthracis, Mycobacterium tuberculosis, and vibrio cholerae as etiologies of anthrax, tuberculosis, and cholera, respectively. Since then, these postulates have been used to determine the causal relationship between an organism and a clinical disease or syndrome, including HIV and AIDS, as well as severe acute respiratory syndrome (SARS)–associated coronavirus with SARS.

However, there are several limitations to Koch's postulates. First, bacteria that are part of the normal flora and do not usually cause disease can become pathogenic in certain situations, such as in an immunocompromised host or with penetration into deep tissue. Second, not all of those infected with these organisms will develop overt clinical disease, as the host immune system may clear the organism or the host may develop only subclinical infection. Third, some organisms are difficult to culture or are not culturable in vitro (eg, Mycobacterium leprae) and may lack an animal model of infection. Finally, something not described in Koch postulates that may be useful in proving causality is if eradication of the pathogen results in cure or impedes disease progression. Keeping these considerations in mind, we will use Koch postulates as a framework to assess the potential role of C pneumoniae in atherogenesis in the discussion below.

**Koch’s Postulate 1: The Pathogen Must be Present in Nearly All Cases of Disease**

**Seroepidemiological Studies**

Lines of evidence supporting Koch’s first postulate include seroepidemiological studies and histopathological detection of the organism in atherosclerotic lesions. The first report of a possible association between C pneumoniae and atherosclerosis was described in 1988: in a case-control study of 40 Finnish men admitted for acute MI (AMI) and 41 matched controls, 68% of the AMI patients had elevated chlamydial immunoglobulin (Ig) G and IgA titers compared with 17% of the controls.

In a separate nested case-control study of 206 patients from the Helsinki Heart Study, the same group noted a 2 to 3 times increased risk in cardiac events among patients with chronic chlamydial infection after adjusting for classic coronary risk factors. A study published by the same authors in 2003 showed that concurrent elevations in CRP in patients with chlamydial seropositivity increased coronary risk from 2- to 3-fold to 4.5-fold.

Danesh et al reviewed 18 seroepidemiological studies (including the 2 described above) in the literature which included 2700 cases and 5000 controls. C pneumoniae antibodies were positively associated with coronary disease in 15 of 16 studies and cerebrovascular diseases in 2 of 2 studies. In the majority of the studies, odds ratios exceeded 2.0. All but 3 of these studies did not adjust for potential confounders. These studies were fairly heterogeneous, varying in the populations studied, the criteria used in defining cardiovascular disease, and the degree of adjustment for confounding variables. However, the overall consistency of their findings suggested a possible association between C pneumoniae and coronary disease.

However, these positive findings have been criticized for a lack of standardized serological methods across studies. The majority of the studies used microimmunofluorescence (MIF) as the serology test. Since it was first developed, this assay has been recognized to be problematic as the test is not standardized and subject to interlaboratory variation. Interpretation of the slides requires an experienced microscopist, and there is a large subjective component to this assay, leading to poor reproducibility. It is difficult to establish or rule out a diagnosis of C pneumoniae infection by a single MIF titer. Especially in children, acute culture-documented infection can occur without a positive MIF. The high prevalence of infection among the adult population also complicates interpretation of MIF results. One study of healthy adults found that 17% of culture and polymerase chain reaction (PCR)–negative individuals had evidence of “acute” infection by MIF.

In addition to the problems inherent to the assay, the abovementioned studies were inconsistent in the serological criteria used to define
past or chronic persistent infection by the MIF assay. IgG and IgA antibody titer cutoffs varied between studies, and in some cases, titers were selected post hoc after data analysis determined which cutoff provided the strongest association. In a few cases, the same investigators used different criteria in different studies. Another source of potential bias is the lack of blinding of the microscopist performing the MIF assay to the disease status of the patient; only 4 of the studies indicated that blinding occurred.

Furthermore, the majority of these earlier studies were cross-sectional, case-control, or retrospective. More recent prospective studies have not confirmed the association between seropositivity for \textit{C pneumoniae} and atherosclerosis. Danesh et al\textsuperscript{23} published a large case-control study nested in a prospective cohort of British men followed over a 16-year period, including 496 men with fatal or nonfatal MI and 989 age- and frequency-matched controls; 40\% of the cases compared with 33\% of the controls had positive \textit{C pneumoniae} titers, yielding an odds ratio for coronary heart disease of 1.66 and 1.22 after adjusting for smoking and socioeconomic status. The same group performed an updated meta-analysis including this study and 14 other prospective studies published through May 2000. These studies included a total of 3169 cases of nonfatal MI or coronary death, and all were adjusted for classic coronary risk factors. The combined odds ratio for coronary disease in these 15 prospective studies was 1.15 (95\% confidence interval 0.97-1.36) compared with 2.0 observed in the earlier meta-analysis published by this group. A recent meta-analysis of 38 seroepidemiological studies between January 1997 and December 2000 also found that the odds ratio for cross-sectional or case-control studies was higher than that of prospective studies (2.0 vs 1.1), with an overall odds ratio of 1.6.\textsuperscript{24}

Although it does not entirely rule out the possibility of an association between \textit{C pneumoniae} and atherosclerosis, this more recent data suggest that a strong causal relationship is less likely.

\textbf{Histopathological Studies}

The presence of \textit{C pneumoniae} in atherosclerotic plaques was first described by Shor et al.\textsuperscript{25} The organism was identified by electron microscopy (EM) and Chlamydia immunoperoxidase staining with genus-specific and species-specific monoclonal antibody in 7 of 7 and 5 of 7 postmortem atheromas, respectively, and in none of the 5 control cases. Gibbs et al\textsuperscript{26} reviewed 17 studies on the detection of \textit{C pneumoniae} in atherosclerotic specimens using a variety of detection techniques including PCR, immunocytochemical staining, immunofluorescence, in situ hybridization (ISH), and direct culture. The organism was identified in 303 (50.8\%) of 597 specimens with atherosclerosis versus only 5 (3.8\%) of 131 specimens without atherosclerosis.

Boman and Hammerschlag\textsuperscript{20} performed an even more extensive review of 43 studies published between 1992 and 2000, including a total of 2644 atherosclerotic specimens. The prevalence of \textit{C pneumoniae} in atheromas varied significantly depending on the particular detection method used. Immunocytochemistry and PCR were the most commonly used; other techniques included direct cell culture, EM, and ISH. A total of 336 (49.7\%) of 676 specimens were positive by immunostaining using Chlamydia genus-specific antibody, and 202 (45.6\%) of 443 specimens were positive by immunostaining using \textit{C pneumoniae}-specific antibody. EM gave positive results in 38 (39.2\%) of 97 specimens. The percentage positive by PCR was significantly less, 558 (24.3\%) of 2294 specimens, whereas direct culture yielded a prevalence of only 7.3\% (33/451 specimens), and ISH was positive in only 1 of 60 specimens examined.

Direct culture of the organism is the gold standard; however, \textit{C pneumoniae} is difficult to grow in cell culture, making this a very insensitive test and reflecting 1 of the limitations of Koch postulates. The lack of a proper “gold standard” makes interpretation of these results difficult, although the prevalence of the organism in atheromas may be as high as 50\%. However, if only those specimens that tested positive by 2 or more independent techniques were considered true positives, the prevalence was reduced significantly. Out of the 502 specimens analyzed by 2 or more \textit{C pneumoniae} techniques, only 76 (16.7\%) tested positive on at least 2 assays.
Koch’s Postulate 2: The Pathogen Must be 
Isolated from the Diseased Host and Grown 
in Culture

Although recovery of *C pneumoniae* by direct 
culture is difficult, a number of groups have 
isolated the organism from atherosclerotic 
plaque. *C pneumoniae* was first isolated by direct 
culture in 1996 from the atheroma of a patient 
with severe coronary disease. 27 Maas et al 28 
isolated viable *C pneumoniae* from 16% (11/70) 
of atheromas from patients undergoing coronary 
bypass surgery, with half of these isolates capable 
of being permanently propagated by serial sub-
cultures. The organism was not found in any of 
the nonatherosclerotic control samples. However, 
1 group failed to identify any *C pneumoniae* in 
patients undergoing atherectomy.29

Koch’s Postulate 3: The Disease Must be 
Reproduced when the Culture is Inoculated 
into the Healthy Host

There have been no studies that have carried out 
Koch’s third postulate exactly as described, for 
example, isolated the organism from a diseased 
host, grown it in culture, and subsequently 
inoculated the cultured isolate into a healthy 
host, causing atherosclerotic disease. However, 
there have been a number of animal studies that 
have shown that inoculation of *C pneumoniae* into 
a healthy host leads to atherosclerotic disease 
compared with uninfected control animals. Fong 
et al 30 and Laitinen et al 31 demonstrated that 
intranasal inoculation of *C pneumoniae* into New 
Zealand White (NZW) rabbits fed a normal diet 
led to the formation of atherosclerotic plaque 
compared with uninfected control NZW rabbits. 
These changes were observed after 1 to 2 weeks 
in one study and 5 to 7 weeks in the other. A few 
animals were noted to have lesions containing 
foamy macrophages and spindle cell proliferation 
of smooth muscle cells.

Moazed et al 32 found that intranasal inoculation of 
*C pneumoniae* into apolipoprotein 
E–deficient mice (a hyperlipidemic mouse model) fed a normal diet accelerated the progression 
of atherosclerotic lesions compared with uninfected control mice. Lesion area in the inner 
curvature of the aortic arch was 2.4 and 1.6 times 
greater at 16 weeks and 20 weeks, respectively, in 
the infected compared with the control mice.

Muhlstein et al 33 randomized 30 NZW rabbits 
fed a cholesterol-enhanced diet to receive intra-
nasal inoculations of *C pneumoniae* or saline. 
Each group was then randomized to a 7-week 
course of azithromycin or no therapy. Maximal 
intimal thickening and plaque area index were 
significantly greater in infected untreated rabbits 
compared with uninfected controls. Maximal 
intimal thickening and plaque area index in 
infected treated rabbits and in uninfected con-
trols did not differ.

Koch’s Postulate 4: The Organism Must be 
Recovered from the Experimentally 
Infected Host

In the animal studies described above, *C pneu-
moniae* could be demonstrated in the vessel walls by 
immunohistochemical staining and by direct 
culture after intranasal inoculation.

A Model of Pathogenesis

There are several ways in which *C pneumoniae* 
may contribute to the pathogenesis of athero-
sclerotic disease. 16 First, *C pneumoniae* may 
cause the initial injury, inducing the atheroscle-
rotic process. Second, the organism may accel-
erate the progression of preexisting disease. 
Third, it may contribute to a disease complica-
tion, such as plaque rupture and MI. Finally, it 
may be an innocent bystander that persists in 
vascular cells but does not contribute to any 
pathological abnormality.

A proposed model of pathogenesis is described 
as follows. First, the organism gains access to the 
vascular endothelium during local infections of 
the respiratory tract. Infected leukocytes may 
disseminate the organism throughout the body 
with activated macrophages carrying the organ-
ism infiltrating the subendothelial layer of the 
coronary arteries. The organism acts as a stimulus 
for chronic inflammation, inducing the produc-
tion of tissue factor, leukocyte adhesion mole-
cules, and inflammatory cytokines including 
tumor necrosis factor (TNF) α, and interleukins 
1, 2, and 6. 26 TNF-α has been observed to 
increase the expression of leukocyte adhesion 
molecules 34 and inhibit lipoprotein lipase, lead-
ing to altered lipid metabolism and accumulation 
of triglycerides in the bloodstream. 35
expression of adhesion molecules promotes leukocyte adherence, migration, and intimal inflammation. These inflammatory cytokines together act to stimulate fibroblast and smooth muscle cell proliferation. They also promote a hypercoagulable state by activating platelets, increasing hepatic synthesis of acute-phase proteins, and inducing tissue factor factor release from the endothelium with activation of the coagulation cascade. Bacterial lipopolysaccharide antigen acts as a potent stimulus for macrophage activation. In addition, it binds to low-density lipoprotein, which could modify the lipoprotein, making it toxic or immunogenic to endothelial cells, and subsequently lead to foam cell formation. Persistent growth of the organism in situ leads to a state of chronic inflammation, platelet activation, vasospasm, and thrombosis.

* C pneumoniae may contribute to atherosclerosis via antigenic mimicry and autoimmunity. Chlamydial heat shock protein 60 (cHsp60) has been isolated in macrophages within atheromatous tissue, colocalizing with its homolog, human hsp. A potential mechanism of atherogenesis that has been suggested is that persistent infection with *C pneumoniae* through the expression of cHsp60 may trigger an autoimmune reaction against human hsp. cHsp60 has also been observed to activate macrophages stimulating TNF-α and matrix metalloproteinase expression, which may contribute to plaque weakening and subsequent rupture.

### Clinical Trials

Although not described in Koch’s original postulates, other lines of evidence that may be useful in determining the role of *C pneumoniae* in the pathogenesis of coronary disease include data from secondary prevention trials in human beings. If *C pneumoniae* is involved in atherogenesis, then presumably, it can be viewed as a

| Trial         | Population                                      | Size | Follow-up | Regimen (vs Placebo) | End Points | Results                                                                 |
|--------------|-------------------------------------------------|------|-----------|----------------------|------------|-------------------------------------------------------------------------|
| Gupta et al38 | Post-MI patients seropositive for Cp antibodies | 60   | 18 mo     | Azithromycin 500 mg x 3-6 d | Composite CV events, Cp antibody titers | ↓ CV events, ↓ antibody titers with Rx |
| ROXIS49      | Unstable angina patients                        | 202  | 1, 6 mo   | Roxithromycin 150 mg bid x 30 d | Composite CV events, Cp antibody titers, CRP levels | ↓ CV events at 1 and 6 mo,* no change in antibody titers, ↓ CRP levels |
| ACADEMIC50   | CAD patients seropositive for Cp antibodies     | 302  | 6 mo, 2 y | Azithromycin 500 mg x 3 d, then 500 mg/wk x 3 mo | Composite CV events, inflammatory markers | No difference in CV events, ↓ CRP and IL-6 at 6 mo |
| ISAR-344     | Coronary-stented patients seropositive for Cp antibodies | 1010 | 1 y       | Roxithromycin 300 mg every day x 28 d | Rate of restenosis, MI, CV death | No difference for restenosis, MI or CV death, but ↓ restenosis in patients with high Cp titers |
| CLARIFY41    | Unstable angina/ NQWMI patients                 | 148  | 3 mo, median 555 d | Clarithromycin 500 mg every day x 3 mo | Composite CV events during treatment and follow-up | ↓ CV events at 3 mo and during follow-up |
| WIZARD42     | Post-MI patients seropositive for Cp antibodies | 7747 | 2.5 y     | Azithromycin 600 mg 4 times a day x 3 d, 600 mg/wk x 11 wk | Composite CV events | Trend toward ↓ CV events (P = NS); possible early benefit |

*Cp* indicates *C pneumoniae*; CV, cardiovascular; CRP, C-reactive protein. Composite CV event reflects a combination of at least 3 of the following clinical end points: fatal/nonfatal MI, unstable angina, unplanned revascularization, stroke, or all-cause mortality. All of these studies included cardiovascular death as an end point.

*The decrease in CV events was only statistically significant at 1 month, although the difference persisted at 6 months.
modifiable risk factor treatable with antibiotic therapy in the same way hyperlipidemia is managed with lipid-lowering agents. Completed clinical trials are summarized in Table 2 and ongoing trials in Table 3.

Gupta et al.38 published the first randomized, double-blinded, placebo-controlled secondary prevention trial of 60 stable post-MI male patients known to be seropositive for C pneumoniae. Subjects were randomized to receive a 3- to 6-day course of azithromycin or placebo. After 18 months of follow-up, the event rate was 8% in the treatment group compared with 28% in the placebo group, a statistically significant reduction, albeit with very small numbers.

The Roxithromycin in Non-Q-Wave Coronary Syndromes (ROXIS) study39 was a secondary prevention trial of 202 patients with unstable angina or non-Q-wave myocardial infarction (NQWMI) randomized to receive a 1-month course of roxithromycin or placebo. Of note, C pneumoniae seropositivity was not used as an entry criterion. There was a statistically significant reduction in recurrent cardiovascular events with 2% of the treatment group compared with 9% of the placebo arm having the triple end point of recurrent angina, AMI, or death. After 6 months of follow-up, this difference persisted, albeit with very small numbers.

The Azithromycin in Coronary Artery Disease: Elimination of Myocardial Infection with Chlamydia (ACADEMIC) trial39 was a secondary prevention trial of 302 coronary patients who were seropositive for C pneumoniae. They were randomized to receive a 3-month course of azithromycin or placebo. After 6 months of follow-up, there was a statistically significant reduction in C-reactive protein and IL-6 compared with placebo; however, no difference in clinical events (fatal/nonfatal MI, unstable angina, unplanned coronary revascularization, stroke) or antibody titers was observed between the 2 groups. After 2 years of follow-up, there continued to be no difference in clinical end points.

The Clarithmyocin in Acute Coronary Syndrome Patients in Finland (CLARIFY) study41 demonstrated a reduction in cardiovascular events in patients with unstable angina or NQWMI after a 3-month treatment period with clarithromycin initiated at the time of presentation, compared with placebo. Over a median period of 555 days, treated patients had a relative risk ratio of 0.49 for a cardiovascular event.

The Weekly Intervention with Zithromax for Atherosclerosis and its Related Disorders (WIZARD) trial42 is the largest clinical trial published to date. It included 3300 patients with a prior MI and positive serologies who were randomized to receive placebo or azithromycin 600 mg 4 times a day for the first 3 days followed by 600 mg once a week for 11 weeks. Although there was a nonsignificant early treatment benefit, this was not sustained throughout the entire 3-year follow-up period.

Brassard et al.43 conducted a nested case-control study of 30,000 subjects status post-MI and noted a nonstatistically significant reduction in risk of acute MI in patients receiving a prescription for antichlamydial antibiotics in the preceding 3 months. The Intraconary Stenting and Antibiotic Regimen (ISAR-3) study44 of poststen patients found that although there was no overall difference in the rate of angiographic restenosis in patients receiving roxithromycin compared with placebo, those patients with high

| Trial      | Population                     | Size  | Follow-up | Regimen (vs Placebo)          | End Points               |
|------------|--------------------------------|-------|-----------|-------------------------------|-------------------------|
| ACES46     | Stable CAD patients            | 4000  | 4 y       | Azithromycin 600 mg every wk × 1 y | Composite CV events     |
| MARBLE     | Patients awaiting CABG         | 1200  | -         | Azithromycin                 | Composite CV events     |
| PROVE-IT46 | Acute coronary syndrome        | 4000  | 2 y       | Gatifloxacin ± statin         | Composite CV events     |
| ANTIBIO    | Acute MI                       | 872   | -         | Roxithromycin                 | Composite CV events     |

CABG indicates coronary artery bypass graft; ACES, Azithromycin and Coronary Events; MARBLE, Might Azithromycin Reduce Bypass List Events; PROVE-IT, Pravastatin or Atorvastatin Evaluation and Infection Therapy; ANTIBIO, Antibiotic Therapy After Acute Myocardial Infarction.
C pneumoniae antibody titers who received the antibiotic had a significantly lower restenosis rate compared with those who did not.

The results of these clinical trials with respect to the potential role of antibiotics in the management of coronary artery disease are inconclusive. These studies varied in a number of ways, including the patient population selected, C pneumoniae seropositivity, the duration of follow-up, the antibiotic used, dosage, and the duration of antibiotic treatment. Patients with stable coronary disease differ from those presenting with an acute coronary syndrome. Patients with unstable plaque may have a higher infective burden or be more likely to have active infection and thus may benefit more directly from antibiotic therapy. Two of the published trials, ROXIS and CLARIFY, involved patients with unstable angina or NQWMI, and both demonstrated a statistically significant reduction in cardiovascular events with treatment. The ACADEMIC and WIZARD trials, conducted in patients with relatively stable coronary disease, yielded negative results. One potential explanation for this disparity is that the organism may remain in its inactive nonreplicating form in stable atherosclerotic plaque, making it less susceptible to the effects of antibiotics.

Areas of Uncertainty and Future Directions

Does C pneumoniae play a role in coronary artery disease? Although the 4 traditional criteria in Koch postulates are not entirely fulfilled, there is evidence to suggest that the organism may be more than simply an innocent bystander.

Seroepidemiological data have provided modest support for an association between atherosclerosis and C pneumoniae, although more recent prospective studies have not been as convincing. When interpreting these findings as well as the findings of clinical trials, it is important to consider the limitations of current serological tests, which are unable to distinguish between previous exposure and active persistent endovascular infection. Several studies have shown that positive serology does not necessarily correlate with presence of the organism in atheroma. Although serologies may be used as a screening test, we lack a gold standard that is able to confirm the presence of active infection in the vascular wall. In the same way that cholesterol levels can be accurately tested to evaluate for hyperlipidemia as a potential modifiable risk factor, we need a sensitive and standardized means of identifying active endovascular infection. PCR to detect C pneumoniae DNA in peripheral blood mononuclear cells may be a better marker of persistent infection and measure of the effect of antichlamydial therapy.

Although the organism is not present in all atheromatous plaque (Koch’s first postulate), histopathological data suggest that the organism has a tropism for atherosclerotic vascular tissues, with viable organism being cultured from these tissues (Koch’s second postulate). However, the difficulty in culturing the organism in vitro and, as described above, the absence of a surrogate marker of active infection pose a diagnostic challenge to determine the presence of an active endovascular infection. Data from animal models provide support for Koch’s third postulate as inoculation of the organism into a healthy host has been observed to cause atherosclerotic disease or increase the progression of atherosclerotic lesions in those with preexisting disease. In these same animal studies, after inoculation with the organism, the presence of C pneumoniae in the vessel walls has been confirmed (Koch’s fourth postulate.)

Because of the limitations of Koch’s postulates in determining an association between C pneumoniae and coronary disease, data from clinical trials have become of paramount importance. A positive clinical trial would provide additional evidence in favor of a causal relationship, whereas a negative trial would suggest the opposite. However, results from current antibiotic clinical trials are inconclusive, and the effectiveness of therapy may vary between different subsets of patients as seen in the ROXIS and CLARIFY studies compared with the ACADEMIC and WIZARD trials. Patients who present with acute coronary syndrome may derive more direct benefit from antibiotic therapy than those with stable coronary disease. Active chlamydial infection may be involved in plaque rupture, and antibiotics may be an important initial therapy in those presenting with acute coronary syndrome in the same way that aspirin, antiplatelet agents, β-blockers, and statins are...
used. The results of the PROVE-IT and ACES trials and other ongoing large-scale clinical trials (Table 3) will be important in clarifying the potential role for antibiotics in the treatment of patients with acute coronary syndrome and coronary disease, respectively.46

There are several other important issues raised by these clinical trials. The metabolically inactive, persistent form of the organism may make its eradication difficult, raising the question of the optimal duration of antibiotic therapy. A longer duration of therapy may be needed to provide bactericidal effects against the organism. If these trials demonstrate a positive treatment benefit, we will need to weigh these benefits against the risk of increasing antimicrobial resistance. An additional area of uncertainty is the degree to which the anti-inflammatory effects of antibiotics account for any of the benefit seen in cardiovascular outcomes.

**Addendum**

Since this manuscript was written, the result of the ANTIBIO and PROVE-IT trial have been released. The ANTIBIO study examined over 800 acute MI patients randomized to roxithromycin or placebo for 6 weeks and found no difference in mortality or other secondary end points between the two groups at 12 months. The PROVE-IT study was a trial of over 4000 patients admitted with an acute coronary syndrome and did not show any cardiovascular benefit associated with long-term (2 years) treatment with azithromycin. The lack of any benefit seen in these trials suggests that there is currently no role for long-term antibiotic treatment (at least with the current anti-chlamydial therapies available) in the management of coronary artery disease.

**References**

1. Frothingham C: The relation between acute infectious diseases and arterial lesions. Arch Intern Med 8:153-162, 1911
2. Minick CR, Fabricant CG, Fabricant J, et al: Atherosclerosis induced by infection with a herpesvirus. Am J Pathol 96:673-706, 1979
3. Grattan MT, Moreno-Cabral CE, Starnes VA, et al: Cytophagalovirus infection is associated with cardiac allograft rejection and atherosclerosis. JAMA 261:3561-3566, 1989
4. Grattan MT: Accelerated graft atherosclerosis following cardiac transplantation: Clinical perspectives. Clin Cardiol 14:16-20, 1991
5. McDonald KM, Rector TS, Braunlin EA, et al: Association of coronary artery disease in cardiac transplant recipients with cytophagalovirus infection. Am J Cardiol 64:359-362, 1989
6. Zhou YF, Leon MB, Waclawiw MA, et al: Association between prior cytophagalovirus infection and the risk of restenosis after coronary atherectomy. N Engl J Med 335:624-630, 1996
7. Sotlie RD, Nieto FJ, Adam E, et al: A prospective study of cytophagalovirus, herpes simplex virus 1, and coronary heart disease: The Atherosclerosis Risk in Communities (ARIC) study. Arch Intern Med 160:2027-2032, 2000
8. Ridker PM, Hennekens CH, Stampfer MJ, et al: Prospective study of herpes simplex virus, cytophagalovirus, and the risk of future myocardial infarction and stroke. Circulation 98:2796-2799, 1998
9. Siscovick DS, Schwartz SM, Corey L, et al: Chlamydia pneumoniae, herpes simplex virus type 1, and cytophagalovirus, and incident myocardial infarction and coronary heart disease death in older adults: The cardiovascular health study. Circulation 102:2335-2340, 2000
10. Danesh J, Collins R, Peto R, et al: Chronic infections and coronary heart disease: Is there a link? Lancet 350:430-436, 1997
11. Pasceri V, Cammarota G, Patti G, et al: Association of virulent Helicobacter pylori strains with ischemic heart disease. Circulation 97:1675-1679, 1998
12. Espinola-Klein C, Rupprecht HJ, Blankenberg S, et al: Impact of infectious burden on extent and long-term prognosis of atherosclerosis. Circulation 105:15-21, 2002
13. Rupprecht HJ, Blankenberg S, Bickel C, et al: Impact of viral and bacterial infectious burden on long-term prognosis of patients with coronary artery disease. Circulation 104:25-31, 2001
14. Kiechl S, Egger G, Mayr M, et al: Chronic infections and the risk of carotid atherosclerosis: Prospective results from a large population study. Circulation 103:1064-1070, 2001
15. Kuo CC, Jackson LA, Campbell LA, et al: Chlamydia pneumoniae. Clin Microbiol Rev 8:451-461, 1995
16. Campbell LA, Kuo CC, Grayston JT, et al: Chlamydia pneumoniae and cardiovascular disease. Emerg Infect Dis 4:571-579, 1998
17. Saikku P, Leinonen M, Mattila K, et al: Serologic evidence for an association of a novel Chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. Lancet 2:983-986, 1988
18. Saikku P, Leinonen M, Tenkanen L, et al: Chronic Chlamydia pneumoniae infection as a risk factor for coronary heart disease in the Helsinki Heart Study. Ann Intern Med 116:273-278, 1992
19. Huittinen T, Leinonen M, Tenkanen L, et al: Synergistic effect of persistent Chlamydia pneumoniae infection, autoimmunity, and inflammation on coronary risk. Circulation 107:2566-2570, 2003
20. Boman J, Hammerschlag MR: *Chlamydia pneumoniae* and atherosclerosis: Critical assessment of diagnostic methods and relevance to treatment studies. Clin Microbiol Rev 15:1-20, 2002
21. Kutlin A, Roblin PM, Hammerschlag MR, et al: Antibody response to *Chlamydia pneumoniae* infection in children with respiratory illness. J Infect Dis 177:720-724, 1998
22. Hyman CL, Roblin PM, Gaydos CA, et al: Prevalence of asymptomatic nasopharyngeal carriage of *Chlamydia pneumoniae* in subjectively healthy adults: Assessment by polymerase chain reaction–enzyme immunoassay and culture. Clin Infect Dis 20:1174-1178, 1995
23. Danesh J, Whincup P, Walker M, et al: *Chlamydia pneumoniae* IgG titres and coronary heart disease: Prospective study and meta-analysis. BMJ 321:208-213, 2000
24. Bloemenkamp DG, Mali WP, Visseren FL, et al: Meta-analysis of sero-epidemiologic studies of the relation between *Chlamydia pneumoniae* and atherosclerosis: Does design influence results? Am Heart J 145:409-417, 2003
25. Kutlin A, Roblin PM, Hammerschlag MR, et al: Antibody response to *Chlamydia pneumoniae* infection in children with respiratory illness. J Infect Dis 177:720-724, 1998
26. Hyman CL, Roblin PM, Gaydos CA, et al: Prevalence of asymptomatic nasopharyngeal carriage of *Chlamydia pneumoniae* in subjectively healthy adults: Assessment by polymerase chain reaction–enzyme immunoassay and culture. Clin Infect Dis 20:1174-1178, 1995
27. Danesh J, Whincup P, Walker M, et al: *Chlamydia pneumoniae* IgG titres and coronary heart disease: Prospective study and meta-analysis. BMJ 321:208-213, 2000
28. Bloemenkamp DG, Mali WP, Visseren FL, et al: Meta-analysis of sero-epidemiologic studies of the relation between *Chlamydia pneumoniae* and atherosclerosis: Does design influence results? Am Heart J 145:409-417, 2003
29. Shor A, Kuo CC, Patton DL, et al: Detection of *Chlamydia pneumoniae* in coronary arterial fatty streaks and atheromatous plaques. S Afr Med J 82:158-161, 1992
30. Gibbs RG, Carey N, Davies AH, et al: *Chlamydia pneumoniae* and vascular disease. Br J Surg 85:1191-1197, 1998
31. Ramirez JA: Isolation of *Chlamydia pneumoniae* from the coronary artery of a patient with coronary atherosclerosis. Ann Intern Med 125:979-982, 1996
32. Maas M, Bartels C, Engel PM, et al: Endovascular presence of viable *Chlamydia pneumoniae* is a common phenomenon in coronary artery disease. J Am Coll Cardiol 31:827-832, 1998
33. Weiss SM, Roblin PM, Gaydos CA, et al: Failure to detect *Chlamydia pneumoniae* in coronary atheromas of patients undergoing atherectomy. J Infect Dis 173:957-962, 1996
34. Fong IW, Chiu B, Viira E, et al: Rabbit model for *Chlamydia pneumoniae* infection. J Clin Microbiol 35:48-52, 1997
35. Laitinen K, Laurila A, Pyhala L, et al: *Chlamydia pneumoniae* infection induces inflammatory changes in the aortas of rabbits. Infect Immun 65:4832-4835, 1997
36. Moazed TC, Campbell LA, Rosenfeld ME, et al: *Chlamydia pneumoniae* infection accelerates the progression of atherosclerosis in apolipoprotein E–deficient mice. J Infect Dis 180:238-241, 1999
37. Muhlestein JB, Anderson JL, Hammond EH, et al: Infection with *Chlamydia pneumoniae* accelerates the development of atherosclerosis and treatment with azithromycin prevents it in a rabbit model. Circulation 97:633-636, 1998
38. Gurfinkel E, Bozovich G, Beck E, et al: Treatment with the antibiotic roxithromycin in patients with acute non–Q-wave coronary syndromes. The final report of the ROXIS Study. Eur Heart J 20:121-127, 1999
39. Sinisalo J, Mattila K, Valtonen V, et al: Effect of 3 months of antimicrobial treatment with clarithromycin in acute non–Q-wave coronary syndrome. Circulation 105:1555-1560, 2002
40. O’Connor C, Dunne MW, Pfeffer MA, et al: Azithromycin for the secondary prevention of coronary heart disease events. The WIZARD study: A randomized controlled trial. JAMA 290:1459-1466, 2003
41. Brassard P, Bourgault C, Brophy J, et al: Antibiotics in primary prevention of myocardial infarction in elderly patients with hypertension. Am Heart J 145:E20, 2003
42. Neumann F, Kastrati A, Miettke T, et al: Treatment of *Chlamydia pneumoniae* infection with roxithromycin and effect on neointimal proliferation after coronary stent placement (ISAR-3): A randomized, double-blind, placebo-controlled trial. Lancet 357:2085-2089, 2001
43. Nghe J, Anand V, Gupta S, et al: *Chlamydia pneumoniae* and atherosclerosis—what we know and what we don’t. Clin Microbiol Infect 8:2-13, 2002
44. Gelfand EV, Cannon CP: Antibiotics for secondary prevention of coronary artery disease: An ACES hypothesis but we need to PROVE IT. Am Heart J 147:202-209, 2004