Is atherosclerosis an infectious disease?

**ABSTRACT**

Atherosclerotic coronary artery disease is multifactorial, but several lines of evidence implicate infection as a potential contributing factor. *Chlamydia pneumoniae* has the most compelling data, with *Helicobacter pylori* and cytomegalovirus also implicated. Clinical trials of antibiotics to decrease coronary events are underway. Until the results are available, however, we advise against prescribing antibiotics for this purpose.

**KEY POINTS**

Atherosclerosis is a specialized inflammatory response involving monocytes and T lymphocytes. Inflammatory mediators such as C-reactive protein and fibrinogen are elevated in infectious diseases and associated mechanistically with atherosclerosis.

Some studies suggest that the risk of coronary artery disease increases with the total infectious load, ie, the total number of different pathogens a person has been exposed to.

Although several small trials showed that a short course of a macrolide antibiotic could reduce coronary events, there is reason for caution; in theory, such therapy could actually increase risk.

Disclosure: This paper discusses treatment that is "off label," ie, not FDA-approved.
ic cardiovascular disease. Most recent studies focus on Chlamydia pneumoniae and cytomegalovirus (CMV), though other infectious agents have also been investigated.6–8 Research suggests multiple possible mechanisms and associations by which infectious agents may facilitate atherosclerotic development and progression.5

■ VIRCHOW AND OSLER THOUGHT OF IT FIRST

Essential groundwork in this field was laid in the 1800s, when Virchow9 reported on the inflammatory nature of atherosclerotic lesions in the course of detailed pathologic evaluation.

Osler's 1908 Textbook of Medicine10 commented, “Experimental production of arteriosclerosis by the various bacterial toxins afford an explanation of this gradual production of sclerosis in the chronic infections.” Many recent reviews have revisited this topic7,8; background detail and many interesting though unproven theories are found in these papers.

■ COXSACKIE B VIRUS

In experiments in mice and monkeys in the 1960s, coxsackie B virus became the first infectious agent noted to produce acute coronary arteritis.11 A few small human case series noted a striking prevalence of seroconversion to coxsackie B at the time of acute myocardial infarction (MI), but prospective studies did not support this association.12–14

■ HERPESVIRUSES

In the mid-1970s, it was noted that Marek disease herpesvirus (MDV), a herpesvirus of fowl, could induce large-vessel occlusive atherosclerotic lesions in chickens. These lesions closely resembled chronic atherosclerotic lesions in humans.

Minick et al15 and Fabricant et al16 conducted an experiment in chickens in which some were deliberately infected with MDV, some were fed a diet high in cholesterol, some received both interventions, and some received neither. The MDV-infected chickens had dramatically more atherosclerotic lesions than did the untreated chickens or those fed the high-cholesterol diet but not infected. In addition, cholesterol feeding had a significant enhancing effect on the prevalence of large coronary artery lesions, consistent with the hypothesis that atherosclerotic cardiovascular disease is multifactorial.

Yet studies of herpesvirus type 1 and type 2 in human atherosclerosis have been unrevealing,17 even though both can infect and grow within human endothelial cells in vitro.18,19

■ CYTOMEGALOVIRUS

CMV is a member of the herpesvirus family that can grow in endothelial and smooth muscle cells. Studies evaluating the potential role of CMV in atherosclerosis were conducted as early as 1983.20 In a more recent study, Zhou et al21 noted a dramatically higher incidence of restenosis after atherectomy and angioplasty in patients who were CMV-seropositive than in those who were CMV-seronegative, 43% vs 8% (P = .002).

Restenosis appears to be largely mediated by smooth muscle proliferation, which is part of the process of atherosclerosis. When CMV infects vascular smooth muscle cells it inhibits p53, a cellular protein that normally regulates smooth muscle proliferation.22 By inhibiting the inhibitor, CMV is believed to promote clonal smooth muscle cell proliferation in coronary restenosis lesions.23

Most studies relating CMV to atherosclerosis evaluated restenosis after angioplasty, but other recent studies did not find an association between angioplasty, CMV, and restenosis.24–27 Angioplasty alone without atherectomy may create a different local effect, which might account for the varied results.

CMV is also thought to play a role in accelerated atherosclerotic coronary disease after heart transplantation,28,29 a leading cause of death in this patient population.28 A recent post-hoc analysis of a study of ganciclovir to control CMV after transplantation found that ganciclovir recipients had a somewhat lower incidence of post-transplantation atherosclerotic disease than did placebo recipients (43% vs 60%, P < .1).29
**Chlamydia pneumoniae**: Perpetrator or innocent bystander in coronary artery disease?

Several lines of evidence implicate *C. pneumoniae* as contributing to coronary artery disease, although formal proof is difficult to establish.

Macrophages infiltrate the subendothelial layer of the coronary arteries and contribute to inflammation and plaque formation.

**Evidence that *C. pneumoniae* plays a role:**
- It can be detected in atherosclerotic plaque
- It can infect endothelial and smooth muscle cells in vitro
- It can induce or promote atherosclerosis in animals after respiratory tract inoculation
- Infection of macrophages with *C. pneumoniae* facilitates their transformation into foam cells, which are part of the cascade of atherosclerotic lesions

*C. pneumoniae* is a common pathogen of the upper and lower respiratory tracts. Macrophages engulf *C. pneumoniae* but do not kill it, and can carry it to distant sites in the body.
**CHLAMYDIA PNEUMONIAE**

*C. pneumoniae* is an obligate intracellular gram-negative organism capable of chronic or persistent infection. It is a recognized cause of acute upper and lower respiratory infection, and its seroprevalence increases with age.30,31

Of the various organisms studied as possible causes of coronary artery disease, *C pneumoniae* has the most evidence in its favor.

**Evidence that *C pneumoniae* contributes to atherosclerosis**

Koch’s postulate states that to prove that a given organism causes a given disease, one has to do four things:

- Observe the organism in every case of the disease
- Isolate the organism from a subject with the disease and grow it in culture
- Inoculate the culture into a susceptible animal and observe that this reproduces the disease
- Observe and recover the organism from the experimentally diseased animal.

Although we cannot fully meet Koch’s postulate in a multifactorial disease such as atherosclerosis, a number of lines of evidence support the hypothesis that *C pneumoniae* contributes to the disease (FIGURE 1).

**MI patients have a high prevalence of infection.** In 1988, while evaluating a serologic assay for *C pneumoniae*, Saikku et al32 in Finland noted a highly statistically significant association between the presence of *C pneumoniae* antibodies and prior MI: 27 (68%) of 40 MI patients had elevated chlamydial IgG and IgA titers, compared with only 1 (2%) of 41 control subjects (*P* < .00001). In a similar study in the United States, Kuo et al33 confirmed this seroepidemiologic association, especially among smokers.

**C pneumoniae can be detected in atherosclerotic plaque.** Multiple studies detected *C pneumoniae* in atherosclerotic plaque specimens by a variety of techniques, including immunohistochemistry, polymerase chain reaction (PCR), and electron microscopy.34–36 A recent review summarized 17 studies of cardiovascular arterial specimens and noted that *C pneumoniae* was identified in 303 (50.8%) of 597 specimens with atherosclerotic involvement.37 In contrast, the organism was found in only 5 (3.8%) of 131 coronary artery specimens without atherosclerotic disease.

Laboratory detection of the organism in atherosclerotic plaque does not prove a causal relationship. Nevertheless, Jackson et al38 and Maass et al39 recently recovered *C pneumoniae* organisms, viable and capable of reproducing, in both carotid and coronary artery specimens. Moreover, the paucity of organisms in nonatherosclerotic tissue and other types of macrophage-rich inflammatory lesions suggests an active role for *C pneumoniae* in the complex atherosclerotic process.40

**C pneumoniae and CMV can infect endothelial and smooth muscle cells in vitro,** adding further support to their possible role in atherosclerotic cardiovascular disease. Cholesterol loading makes smooth muscle cells more vulnerable to infection with *Chlamydia* organisms.41

**C pneumoniae is carried through the body by macrophages.** *C pneumoniae*-infected macrophages are capable of infecting endothelial cells,41,42 and PCR testing of peripheral blood monocytes shows *C pneumoniae* in up to 25% of people.43,44 These data suggest how an intracellular pulmonary infection may migrate to a distant vascular site, using circulating macrophages as a kind of Trojan horse.45,46

**Experimental C pneumoniae infection can induce or promote atherosclerosis in animals.**39,47–51 In mice and rabbits, inoculation of *C pneumoniae* into the upper respiratory tract can produce atherosclerotic lesions in the aorta of these animals. In apoE-deficient mice, *C pneumoniae* infection accelerates the progression of atherosclerosis in the aortic arch.52 Normocholesterolemic rabbits develop intimal alterations when infected with *C pneumoniae*.49,53 Muhlestein et al50 showed that rabbits fed a modestly cholesterol-enhanced diet developed accelerated aortic atherosclerosis, which was dramatically reduced by treatment with the macroide antibiotic azithromycin. *C pneumoniae* antigen could be detected in the aortic atheroma in this model, and while treatment ameliorated the accelerated atherosclerosis, *C pneumoniae* antigen was still detected in the atheroma after treatment. It is unclear whether this persistence represented viable quiescent organism or merely antigenic rem-
nants of killed organism. Infection of monocytes with \textit{C} pneumo-
"niae facilitates their transformation into foam cells, which are clearly part of the initi-
ation cascade of atherosclerotic lesions.\textsuperscript{54} Together, these data are consistent with the view of atherosclerosis as a complex disease process with a strong inflammatory component. The idea that inflammation, in addition to being a response to injury, is the overall pathologic process in atherosclerotic lesions would certainly fit with \textit{C} pneumo-
"niae or other chronic infectious agents playing part of the role in this disease process. It remains unproven whether \textit{C} pneumo-
"niae is actively involved in driving this inflammatory process or whether it is merely an “innocent bystander.”

\textbf{HELICOBACTER PYLORI}

A possible association between the chronic infectious agent \textit{Helicobacter pylori} and atherosclerotic coronary vascular disease was first evaluated in 1994.\textsuperscript{55} Reasons for investigating this agent included a known association between low childhood socioeconomic status (a risk factor for \textit{H pylori} acquisition) and coronary artery disease as an adult.\textsuperscript{56} There also is evidence of an association between gastric cancer, peptic ulcer disease, and atherosclerotic cardiovascular disease.\textsuperscript{57} Patients who have \textit{H pylori} antibodies have higher levels of fibrinogen and C-reactive protein, which are both markers associated with clinically relevant atherosclerotic cardiovascular disease. Treatment of \textit{H pylori} (and \textit{C} pneumo-
"niae) reduces fibrinogen levels.\textsuperscript{58}

By itself, seropositivity for \textit{H pylori} seems to have a much weaker association with atherosclerotic coronary artery disease than does seropositivity for \textit{C} pneumo-
"niae, and this avenue of research is waning.

People typically acquire more than one infection in their lifetimes, with more than one organism. Recent work noted an increased odds ratio for the development of atherosclerotic cardiovascular disease in peo-

\begin{table}[h]
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\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{TRIAL} & \textbf{POPULATION} & \textbf{REGINEN$^*$} & \textbf{SIZE} & \textbf{ENROLLMENT} & \textbf{END POINTS} \\
\hline
Preliminary trials & & & & \\
ACADEMIC & Coronary disease$^\dagger$ & Azithromycin & 300 & Closed & Composite$^\dagger$ \\
ROXIS & Peri-MI & Roxithromycin & 202 & Closed & Composite \\
\hline
Ongoing trials & & & & \\
WIZARD & Post-MI$^\ddagger$ & Azithromycin & 7,000 & Closed & Composite$^\dagger$ \\
ACES & Coronary disease & Azithromycin & 4,000 & Closed & Composite$^\dagger$ \\
MARBLE & Awaiting CABG & Azithromycin & 1,200 & Open & Composite$^\dagger$ \\
ANTIBIOS & Post-MI$^\ddagger$ & Roxithromycin & 4,000 & Closed & Composite$^\dagger$ \\
PROVE-IT & Coronary disease & Gatifloxacin $\pm$ statin & 4,000 & Open & Composite$^\dagger$ \\
STAMINA & Peri-MI & Anti-\textit{H pylori} & 600 & Open & Inflammatory markers \\
APRES & Angioplasty & Roxithromycin & 1,000 & Open & Restenosis \\
MUNICH & Angioplasty & Roxithromycin & 1,000 & Closed & Restenosis \\
\hline
\end{tabular}
\caption{Ongoing clinical trials of antibiotics in atherosclerotic cardiovascular disease}
\end{table}

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\textsuperscript{*}All studies have a placebo group \\
Azithromycin dosage is 600 mg once weekly for 3 months, except in ACES, in which the dosage is weekly for 1 year \\
STAMINA compares two anti-\textit{H pylori} regimens: omeprazole + azithromycin + metronidazole vs omeprazole + amoxicillin + metronidazole \\
Roxithromycin dosage is 300 mg daily for 4 (MUNICH) to 6 (ANTIBIOS, APRES) weeks \\
$\dagger$Patients must be seropositive for \textit{C} pneumo-
"niae antibodies \\
$\dagger$Death, MI, revascularization, angina, or other cardiovascular event
\end{flushleft}
倘感染导致冠状动脉疾病，抗生素应减少事件

如果感染导致冠状动脉疾病进展，那么治疗这些感染应减少冠状动脉事件。要评估这一假设，初步的抗生素治疗在人类中的试验已经进行，并且还有更多的试验正在进行（表1）。

初步试验

古普塔等63在美国的一项试验中，筛选了220名在6个月内患过MI的男子，并根据他们的抗体检测结果对C。 pneumoniae进行了分类。80名抗体检测结果最高的男子被随机分为3组：40名接受每日500 mg的阿奇霉素，20名接受安慰剂，其余20名选择不接受药物，并被标记为"对照"组。接下来18个月内，阿奇霉素治疗组的高检测结果比例为8%，而安慰剂组为25%，对照组为30%。8%的比例与未接受治疗的阳性组相当。

《ACADEMIC实验》64在302名C。 pneumoniae阳性患者中，显示了在治疗后6个月中阿奇霉素和安慰剂之间没有差异，但显示了C-反应蛋白和炎症细胞因子 interleukin 1和 interleukin 6的显著下降。

《ROXIS实验》65相反，没有使用C。 pneumoniae阳性作为入选标准。202名患者在经历非Q波MI或不稳定型心绞痛后8天内入组，并随机分为每日两次150 mg的roxithromycin组或安慰剂组。1个月后，治疗组的发病率显著降低，但随着时间的推移，效果有所丧失。

评论。

这些试验的设计差异和结果反映了感染与动脉粥样硬化性心血管疾病之间潜在关联的复杂性，以及对抗生素治疗慢性衣原体感染的最佳方式的不确定性。还有对动物研究中抗生素的抗动脉粥样硬化作用是由于抗菌作用，弱但可测量的抗炎性质，还是两者结合的争议。66–69

正在进行的抗生素作为二级预防的随机试验

在有相当强的流行病学证据、阳性动物研究和初步临床试验结果的基础上，多个大规模的多中心安慰剂对照试验正在进行抗生素用于冠状动脉事件的预防。《WIZARD实验》是一项双盲、安慰剂对照的试验，评估了抗生素的疗效。
cacy of weekly azithromycin on the incidence of coronary artery disease in patients with a remote history of MI (> 6 months) and positive IgG serology of C pneumoniae. Patients in the active-treatment group received azithromycin 600 mg daily for 3 days followed by maintenance therapy of 12 weekly doses. Starting in the fall of 1998, 7,000 patients were enrolled in only 3 months. The study should have the statistical power to detect a 15% reduction in clinical end points (death, MI, need for coronary revascularization, or hospital admission for angina). However, because the estimated annual event rate in this very stable patient population is only 8%, follow-up must continue for 36 months to reach statistical significance. Results are expected in early 2003 (Personal communication; Michael Dunne, MD).

The ACES trial. Perhaps a short course (12 weeks) of antibiotic therapy is insufficient to effectively treat a potentially chronic chlamydial infection. This concept could help explain the loss of effect over time in the ROXIS trial. This potential shortcoming will be addressed in the Azithromycin and Coronary Events Study (ACES). Funded by the National Heart, Lung, and Blood Institute, this study is currently enrolling patients with a history of MI regardless of Chlamydia serology. Patients are being randomly assigned to receive either placebo or a weekly dose of azithromycin for 1 year.

A group of patients is also being enrolled in a substudy using serial blood collection to examine the potential association between serology, immune markers, and response to therapy. Recruitment is complete, and results are expected in late 2003.

Comment. While the WIZARD and ACES trials should help determine whether a short or long course of azithromycin therapy reduces the incidence of coronary events, both trials are targeting similar patients at a very stable stage of atherosclerotic progression. Both studies require patients to be at least 6 months out from the most recent MI or coronary revascularization.

Opinion differs as to whether the association between C pneumoniae and atherosclerosis is due to an effect on the chronic progression of atherosclerosis, activation and destabilization of a fairly advanced plaque, or non-healing of a recently destabilized plaque. If the role of C pneumoniae is in early lesion progression, treating patients with established but stable atherosclerosis may be too late.

On the other hand, if the primary role of treatment is in healing a ruptured plaque, the trials starting 6 months after an acute coronary event may be outside the window in which lesion stabilization can be maximally influenced. Possibly the appropriate time to treat patients with antibiotics is when they present with an acute coronary syndrome of unstable angina or non-Q wave MI caused by a recently inflamed and ruptured plaque. It was this patient population that was targeted in the pilot ROXIS trial.

ANTIBIOS, a larger post-MI placebo-controlled roxithromycin trial, is enrolling 4,000 patients to gain greater statistical significance.

STAMINA, another peri-MI trial underway, will enroll 600 patients, and compare regimens with anti-H pylori activity (a proton pump inhibitor plus azithromycin vs amoxicillin plus metronidazole).

PROVE-IT. Quinolones, like macrolides, also have in vitro activity against chlamydial organisms. At least one placebo-controlled trial underway (PROVE-IT) is evaluating the quinolone antibiotic gatifloxacin. This trial will also randomize patients to receive lipid-lowering (“statin”) drugs or placebo.

The MARBLE study in the United Kingdom is taking advantage of the delay in that country between the time coronary artery bypass grafting is found to be needed and the time it is actually performed. Researchers want to see if azithromycin (vs placebo) has any impact on disease progression preoperatively. At 1,200 patients, and with planned coronary catheterization before surgery, this study should address unique issues not evaluated in the other trials.

The APRES and MUNICH studies are each enrolling approximately 1,000 patients who have undergone angioplasty, randomizing them to receive roxithromycin or placebo. Enrollment in APRES is still open, but MUNICH is already closed. These studies should help address any
potential role for antichlamydial therapy to reduce restenosis.

Smooth muscle proliferation seems to play more of a role in restenosis after angioplasty than in other types of coronary disease, and CMV seems to promote smooth muscle proliferation. However, we are not aware of any anti-CMV drug trials in angioplasty patients.

Can antibiotics prevent first-time acute MI?
A large case-control study in the United Kingdom71 enrolled more than 3,000 patients with a first MI who did not have classic risk factors (hyperlipidemia, hypertension, or diabetes) and compared them with 13,139 matched healthy controls. In the 3 years before the MI, the MI patients were less likely to have been prescribed tetracycline or fluoroquinolone antibiotics for various infections (P < .05 for both comparisons). These agents are active against C pneumoniae. Though macrolide antibiotics also have antichlamydial activity, the MI patients were not less likely to have received macrolides. A possible explanation is that the macrolides probably were used at doses ineffective in treating C pneumoniae infection.

Subsequent studies in the United States did not find an association between a first MI and use of erythromycin, tetracycline, or doxycycline.72

■ LOOKING BACK, LOOKING FORWARD

The trials performed so far support a possible association between infectious pathogens and atherosclerotic cardiovascular disease, but they are far from establishing a cause-effect relationship. Placebo-controlled treatment trials may provide more direct evidence of an association. Overall, the data reinforce the concept that atherosclerotic cardiovascular disease is multifactorial and that inflammatory changes contribute to its progression.

The epidemiologic studies and the preliminary treatment trials do not clearly settle the issue about C pneumoniae’s role in atherosclerotic cardiovascular disease, nor should they encourage widespread application of antibiotic therapy in patients with atherosclerotic cardiovascular disease. Treatment of chronic bacterial pathogens may result in transient immune activation, which may even worsen coronary artery disease.

It is hoped that as ongoing study data emerge, better understanding of pathophysiological associations and the role of anti-infective therapy will guide future treatment of atherosclerosis.

■ REFERENCES

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