

Review Article

Chlamydia pneumoniae (and other infective agents) in atherosclerosis and acute coronary syndromes

How good is the evidence?

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Introduction

It is acknowledged that local and systemic inflammatory processes play an important role in the development and progression of atherosclerotic lesions, and in the pathophysiology of acute coronary syndromes^[1,2]. Established cardiovascular risk factors do not completely explain the epidemiological changes of, and the diverse presentations of, coronary heart disease. This had led to increasing interest in the contribution of certain infectious agents as atherogenic risk factors. The evidence for *Chlamydia pneumoniae* as a potential causative agent is strong, and is largely based on the findings of (1) seroepidemiological studies, (2) examination of atheromatous plaque specimens, (3) in-vitro experiments and animal models and recently, (4) preliminary anti-chlamydial antibiotic intervention studies. This review aims to focus on the scientific evidence of the implication of *C. pneumoniae* (and other infective agents) as a culprit aetiological agent in atherogenesis and coronary heart disease.

Candidate infective agents and atherogenesis

Elegant animal experiments in the 1970s showed that Marek's disease virus (an avian herpesvirus) could produce arterial lesions in chickens infected with the virus^[3]. With concomitant consumption of a cholesterol-enriched diet, the animals developed fibro-fatty lesions, resembling human atherosclerosis. In contrast, vaccination before exposure to the virus prevented lesion formation^[4].

Cytomegalovirus, another common herpes virus, has been linked to some forms of human arterial disease^[5–8]. Compared with controls, elevated antibodies to cytomegalovirus appear to be more common in patients with atherosclerosis^[6]. However, seroepidemiological studies so far have been limited by small sample sizes and with incomplete adjustments made for known confounding variables^[9]. Cytomegalovirus is more strongly associated with coronary artery restenosis following coronary angioplasty/atherectomy^[7] and with the development of accelerated graft vasculopathy seen in heart transplant recipients^[8]. In contrast to native atherosclerosis, this latter form of atherosclerotic disease predominately comprises diffuse smooth muscle proliferation and collagen accumulation^[10].

Several studies have reported an association between seropositivity to *Helicobacter pylori* and coronary heart disease^[11–13]. The earlier investigations suggested that *H. pylori* may lead to an increase in markers of inflammation that contribute to atherothrombosis such as leucocyte count, fibrinogen and C-reactive protein^[13]. More recent, larger studies incorporating prospective data and better defined control subjects have failed to establish an independent link between this infection and coronary heart disease^[14–16]. These studies suggest that lower socio-economic status is a major confounding variable in a relationship between the organism and atherosclerotic disease. Furthermore, a recent meta-analysis of the published sero-epidemiological studies involving some 10 000 patients found no significant correlation between *H. pylori* and a number of inflammatory mediators^[17]. Direct pathological examination of atherosclerotic plaques have also failed to detect any evidence of *H. pylori* DNA^[18]. It is possible that only certain virulent strains of *H. pylori* (i.e. Cag-A positive) have an established association with coronary heart disease, as suggested by a recent case-controlled study^[19], but this needs verification with large prospective studies.

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The role of dental infections^[20] and chronic bronchitis^[21] has also been proposed. The associations, although plausible (via bacterial-induced endothelial cell damage or through indirect mechanisms), need further evaluation.

***Chlamydia pneumoniae* in atherosclerosis and coronary heart disease**

The link between anti-C. pneumoniae antibody and coronary heart disease

Chlamydia pneumoniae is an obligate, intracellular pathogen and a common cause of benign respiratory symptoms^[22]. The first report of an association between *C. pneumoniae* and coronary heart disease came from an observational study by Saikku and co-workers in 1988^[23]. Compared with controls, elevated antibodies to *C. pneumoniae* were detected more commonly in patients with acute myocardial infarction and chronic coronary heart disease. Since this report some 20 independent studies have corroborated the original findings and even after controlling for major confounding factors there exists an independent correlation between a raised anti-*C. pneumoniae* antibody titre and increased prevalence of coronary heart disease^[9]. Despite this consistent serological link between *C. pneumoniae* and different stages of coronary heart disease, there are few prospective studies and the diagnosis of a chronic *C. pneumoniae* infection has been largely indirect and inconsistent. The microimmunofluorescence antibody test used for diagnosing *C. pneumoniae* infection is non-standardized, variable cut-offs have been used to determine seropositivity and an anti-*C. pneumoniae* antibody titre score is highly dependent on the visual skill of the microscopist^[9,24].

Evidence from plaque examination

First reported in 1992^[25], stronger evidence for an association between *C. pneumoniae* and coronary heart disease comes from direct examination of atherosclerotic tissue. *C. pneumoniae* DNA, protein and/or elementary bodies have been identified in diseased human arterial tissue, using molecular, immunohistochemical or other techniques. Several published studies have evaluated the presence of *C. pneumoniae* in pathology samples. In nearly 50% of atheromatous lesions, *C. pneumoniae* was seen in only 5% of samples from non-atheromatous arterial segments^[9]. Because the organism has also been found in body tissue sites other than the coronary arteries (such as stenosed aortic valves^[26], hepatic vessels^[27], and spleen^[28]) has led to the so-called 'innocent bystander hypothesis'. However, in a recent autopsy study, *C. pneumoniae* was detected using PCR and immunocytochemistry in a greater proportion of cor-

onary atheromatous lesions and less frequently in other tissues — suggesting a predilection of the organism for vascular atheromatous tissue^[29].

In 1995, *C. pneumoniae* was eventually cultured from a coronary atheromatous lesion^[30]. This isolation of 'viable' organism has now been reproduced by other investigators^[31–32], and strengthens the implication for a pathogenic role of *C. pneumoniae* in atherosclerosis, and also helps towards fulfilling Koch's postulates for disease causality.

In-vitro evidence and potential mechanisms of damage

In the laboratory, *C. pneumoniae* has been shown to infect and replicate within smooth muscle cells, endothelial cells and macrophages^[33], which are all important components of the atherosclerotic plaque. It is hypothesized that macrophages/monocytes may transport *C. pneumoniae* from the respiratory tract to the coronary arteries. Local infection of the coronary vasculature may create a focus for intimal damage and may promote an inflammatory response. Activation of monocytes/macrophages leads to cytokine production, synthesis of acute phase proteins^[13] and expression of procoagulant markers such as tissue factor^[34], events that all contribute to atherothrombosis. A genetic predisposition for such a chronic *C. pneumoniae* infection to trigger these processes is possible. A combination of male sex HLA DR II genotype 13a or 17, elevated levels of lipoprotein(a) and anti-IgG *C. pneumoniae* antibody titres (>1/256) have been strongly associated with coronary heart disease^[35]. The scenario may be analogous to *C. trachomatis*-induced development of trachoma^[36]. Childhood infection of the conjunctiva with this organism leads to an intense inflammatory infiltrate. Many years later, and in susceptible individuals, scarring of the conjunctiva may occur (perhaps as a result of a hypersensitivity reaction) which eventually leads to blindness.

The temporal sequence of events for a potential *C. pneumoniae* infection leading to atherogenesis, and the relationship between infection and established cardiac risk factors remains unexplained^[24]. Intriguingly, infection is more common in males^[22] and elevated antibodies to *C. pneumoniae* have been associated with an atherogenic lipid profile^[37], hypertension^[38] and smoking^[39]. A correlation has also been found between *C. pneumoniae* and an elevated level of fibrinogen and C-reactive protein in patients with coronary heart disease^[13].

An animal model for C. pneumoniae-induced atherogenesis

Fong *et al.* first showed that rabbits infected with *C. pneumoniae* developed pneumonia and two of six animals also formed fatty streaks and grade III

Table 1 Role of *Chlamydia pneumoniae*, *Helicobacter pylori* and *Cytomegalovirus* in atherosclerosis: lines of evidence

	Cytomegalovirus	<i>Helicobacter pylori</i>	<i>Chlamydia pneumoniae</i>
Seroepidemiology			
atherosclerosis	+	+	+
restenosis	+	-	-
transplant			+
arteriosclerosis	+	-	-
Presence in plaque	+	-	+
'Viable' organism cultured from plaque	-	-	+
Growth in vascular cells in vitro	+	-	+
Atherogenesis in an animal model	+	-	+
Link with inflammatory/thrombotic markers	±	+	+

atherosclerotic lesions in the aorta^[40]. Similarly, Laitinen *et al.* found inflammatory changes, intimal thickening and fibroid plaques resembling atherosclerosis in the aortas of six of nine inoculated rabbits^[41]. Immunohistochemistry for *C. pneumoniae* antigen was positive in all of these animals. Control animals had no signs of atherosclerosis. More recently, Muhlestein and colleagues showed that intranasal *C. pneumoniae* infection accelerated the formation of intimal thickening in the aortas of rabbits given a high-cholesterol diet, but not in a subgroup of animals receiving additional weekly treatment with azithromycin antibiotic^[42]. The findings from these early animal models^[1], although based on small numbers, suggest that *C. pneumoniae* may initiate the development of atherosclerosis. Whether these preliminary experiments are representative of 'natural' *C. pneumoniae* infection in humans requires further investigation.

Acute coronary syndromes

It is clear from the above that chlamydia is found in macrophages in a high proportion of atherosclerotic plaques (Table 1 and Fig. 1). The production of TNF- α and interleukin 1 and 6 and as well as other substances may damage the intima and lead to plaque rupture. It has been suggested that treatment of chlamydial infections with antibiotics could reduce the cytotoxic effects of the macrophage on the intima with a consequent reduction in coronary events.

The rise in C-reactive protein and serum amyloid A seen in patients with acute coronary syndromes and their association with the outcome of such patients provides evidence to support the inflammatory nature of this condition. Liuzzo *et al.* measured C-reactive protein and serum amyloid A as well as a creatinine kinase and cardiac troponin T in 32 patients with chronic stable angina, 31 patients with severe unstable angina and 29 with acute myocardial infarction^[43]. It was found that elevation of C-reactive protein and serum amyloid A protein at the time of hospital admission predicted a

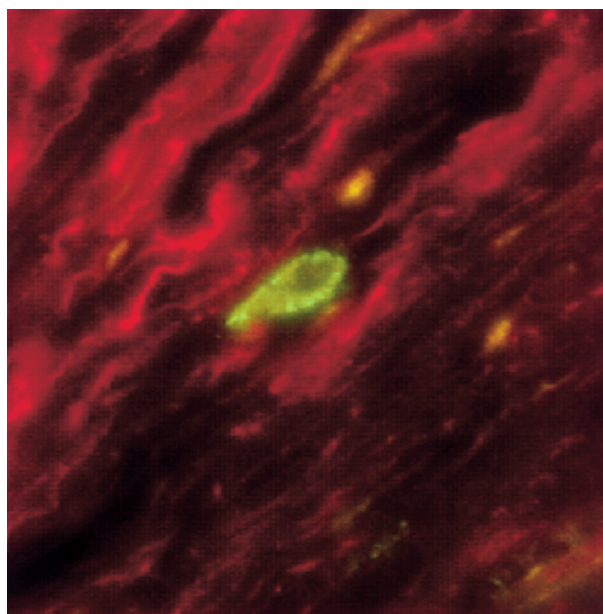


Figure 1 Section of human coronary plaque stained for immunofluorescence with an antibody to the chlamydial capsular antigen. A macrophage contains numerous organisms stained bright green.

poor outcome in patients with unstable angina, independent of elevations of creatinine kinase and troponin T. The findings were further supported by studies from the European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study group in which 2121 patients with angina were investigated; 1030 had unstable angina, 743 stable angina and the remainder atypical chest pain^[44]. All patients underwent coronary angiography and extensive clinical and laboratory assessment at study entry and were followed for 2 years. The risk of coronary events was related to the level of C-reactive protein at study entry, with the frequency of events related to the level of C-reactive protein. These were not patients with myocardial infarction and the acute phase response of C-reactive protein was not felt to be the

result of myocardial necrosis, but rather an inflammatory marker of the underlying disease process.

Pilot antibiotic trials

On the basis of the above theoretical considerations there have been two encouraging pilot trials published so far. In the studies of Gupta and his colleagues, 220 consecutive male survivors of myocardial infarction were screened for anti-*C. pneumoniae* antibodies^[45]. These 213 patients were stratified into three groups viz, those with no detectable antibodies, those with intermediate titres and those who were considered undoubtedly seropositive. Subsequently, patients who were seropositive were randomized to oral azithromycin or placebo (28 patients vs 20 patients). A further 20 patients not recruited into the antibiotic trial, who were markedly serum positive for *C. pneumoniae*, were followed-up. Although only small numbers of patients were included in this study, patients who were seropositive for *C. pneumoniae* had a similar outcome to seropositive patients who were treated with placebo and had a fourfold increased risk of adverse cardiovascular events compared to the seronegative group (confidence interval 1.2 to 15.5 $P=0.03$). In contrast, cardiovascular events in patients receiving azithromycin who were seropositive for *C. pneumoniae* were the same as the seronegative group. At 6 months in the patients participating in the antibiotic trial, the anti-*C. pneumoniae* titre fell to less than 1 in 16 in 43% of the patients receiving azithromycin compared to only 10% of patients taking placebo ($P=0.02$). There was no direct comparison reported between the antibiotic- and placebo-treated patient in terms of cardiovascular events. However, the numbers involved in this antibiotic trial would really be too small to expect to see a difference in cardiovascular outcome. This study was supported by the ROXIS pilot study but more recently the follow-up to the ROXIS study^[46,47] was negative.

In the ROXIS pilot study, 202 patients with unstable angina or non-Q wave myocardial infarction were randomly assigned to receive either roxithromycin 150 mg orally twice daily or placebo. The treatment was for 30 days and the follow-up for 6 months. A statistically significant reduction in cardiac ischaemia death, myocardial infarction and severe recurrent ischaemia at day 31 was seen in the patients treated with roxithromycin. This effect was maintained through to 6 months.

The effects of macrolide therapy in the setting of coronary heart disease has been further tested by additional small studies. 'The Azithromycin in Coronary Artery Disease: Elimination of Myocardial Infection with Chlamydia (ACADEMIC)' Study treated 302 patients with coronary heart disease who had a seropositive reaction to *C. pneumoniae* (IgG titres $\geq 1:16$) to azithromycin (500 mg \cdot day⁻¹ for 3 days, then 400 mg \cdot week⁻¹ for 3 months) or placebo^[47]. At 6 months, compared with the placebo group, those patients receiving azithromycin had a significant fall in

certain markers of inflammation (C-reactive protein and IL-6) but no differences were observed in anti-chlamydia pneumoniae antibody titres of clinical events.

The ACADEMIC study findings contrast with the positive results of the pilot azithromycin study by Gupta *et al.* However, ACADEMIC randomized patients with any (low) level of anti-*C. pneumoniae* antibody titre (rather than those with stable elevated titres). Furthermore, the change in anti-*C. pneumoniae* antibody titre was measured with a diagnostic test (ELISA) different to that used in the screening phase (microimmunofluorescence assay). The number of events reported at 6 months in ACADEMIC is small (9 vs 7), the primary clinical end-point of the study being due at 2 years follow-up.

A study by Torgano *et al.* randomized 84 patients with chronic coronary heart disease and seropositivity to *H. pylori* and/or *C. pneumoniae* to treatment (or no treatment) against these infections^[48]. Those patients with positive anti-*C. pneumoniae* antibody titres were given a 14 day course of macrolide, clarithromycin. These treated patients were shown to have a significant fall in plasma fibrinogen levels and also a significant fall in mean IgG geometric titre level against *C. pneumoniae* (compared with untreated group).

The findings of all the early intervention studies in coronary heart disease indicate that macrolides may exhibit independent, anti-inflammatory properties—in addition to their established anti-chlamydial action. This is also supported by *in vitro* work^[49].

These clinical studies provide an intriguing therapeutic approach to the patient with unstable angina, non-Q wave infarction and recurrent ischaemia following acute myocardial infarction. They lend support to the hypothesis that infection with *C. pneumoniae* is an important pathophysiological mechanism in the development of plaque rupture as well as in the outcome of these patients. Clearly we await the currently ongoing large scale trials before we can consider that antibiotics should be part of the management of acute coronary syndromes.

This paper forms the basis of two lectures to have been given by the authors on the day of Professor Ronnie Campbell's tragic death at the Cardiovascular Forum meeting held in Spain. This work is therefore dedicated to the memory of Ronnie Campbell.

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