# THE ROLE OF CHLAMYDIA PNEUMONIAE IN ATHEROSCLEROSIS

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## IS CARDIOLOGY READY FOR REVOLUTION?

## INTRODUCTION

During the past 15 years several viruses, including herpes simplex, cytomegalovirus, and coxsackie B virus, have been implicated in heart disease.[1-6] Chlamydia pneumoniae is one of the new, emerging infectious agents which was recently linked to atherosclerosis.

The chlamydia are obligate intracellular bacteria characterized by a unique growth cycle and responsible for a wide variety of diseases in man and animals.[7]

Three different species of the genus chlamydia are recognized.

- C. trachomatis
- C. psittaci

And the newly designated species

- C. pneumoniae

Chlamydia pneumoniae is not what you would expect. It is less famous than its cousins which cause sexually transmitted disease (C. trachomatis) or conjunctivitis (C. psittaci), but it is far more widespread and may be far more dangerous.[7-8]

We will all encounter this bacterium sooner or later, most of us more than once. It is commonly spread through coughs and sneezes, causing a flulike respiratory condition that sometimes progresses to pneumonia.[9,10]

A high proportion of adults from different countries are positive for antibodies to C. pneumoniae, implying a high prevalence of these infections.

Several lines of evidence suggest that Chlamydia pneumoniae can make its way into the walls of various blood vessels, linger for years inducing the inflammation and immune reaction that causes heart attacks and strokes.

This does not imply that C. pneumoniae infection is the sole cause of atherosclerosis, or that diet and exercise do not matter. But mounting evidence suggests that the leading cause of death in the western world is to some degree contagious and that common antibiotics might help bring it under control.

*This is how scientists made the connection between a germ*

*and America's leading cause of death.*

The bug first came under suspicion in 1988, when Dr. Saikku and his colleagues[11] published an article in Lancet about the serological evidence of an association of a novel Chlamydia, with coronary heart disease and acute myocardial infarction. In this study they showed that people with coronary artery disease were more likely than healthy control subjects to have circulating IgG and IgM antibodies to C. pneumoniae in their blood.

The second study by the same group[12] published in Annals of Internal Medicine was entitled "Chronic Chlamydia pneumoniae infection as a risk factor for coronary heart disease in the Helsinki Heart Study." In this study unusually high antibody levels turned up in the blood of heart attack victims. While most experts dismissed the Finnish findings, Dr. Grayston at the University of Washington, who during 1981[13] described the association between childhood myocarditis and Chlamydia Trachomatis infection, was intrigued enough to launch his own study and in three different publications documented the same pattern in Seattle that other researchers had seen in Helsinki. Since then eight different research teams from five countries have confirmed these findings.[15-22]

## *Does the Association Between Antibody and Heart Disease Mean Anything?*

The antibody presence tells you only that sometime during his lifetime a person has encountered this pathogen and mounted an immune response. It does not reveal whether Chlamydia is still present or, if so, how it is affecting the body.

With those questions in mind, researchers in the early 90's by using immunocytochemistry, electron microscopy, and especially electron microscopy to look for direct evidence of C. pneumoniae in the blood and clogged blood vessels.

The first piece of evidence came in 1993[23] when researchers spotted a tiny, pear-shaped bacteria after histopathological examination of diseased arteries.

These results were confirmed by another group after examinations and detection of C. pneumoniae's genetic material in 20 out of 36 tissue samples.[24]

Other labs followed, and most of them found bacterial fingerprints or genetic material by one method or another.[24-26]

But similar to Helicobacter pylori and its association with ulcers, only a few paid attention to the relationship between this Chlamydia and diseased arteries until 1994. It was Dr. Summersgill's (of the University of Louisville) efforts which resulted in direct detection of alive and kicking bacteria from a patient's blood vessels. This patient, who was undergoing a heart transplant, without a recent history of respiratory illness, when his coronary artery tissue was placed in a culture dish colonies of C. pneumoniae were detected.[27]

Based on this pioneering work today , no one familiar with the scientific literature denies that Chlamydia pneumoniae is associated with vascular disease. Regardless of their age, sex or nationality, people with sclerotic arteries tend to show signs of infection. Unlike the other infectious agents sometimes shown in both healthy and unhealthy patients, this organism never shows up in truly healthy tissue.

## *Finding The C.pneumoniae at the Crime Scene Does Not Prove That it is a Criminal.*

Scientists have long known that atherosclerosis is an inflammatory disease which affects vessels throughout the body in particular those supporting the heart and the brain. So far no one has shown conclusively that C. pneumoniae causes the damage that leads to heart attack but there are good reasons to believe that it plays an important role in the inflammatory response.

One of the functions of the immune system is to remove fat, cholesterol and other irritants from the vessel walls.

As it is shown by its designation, Chlamydia pneumoniae has been established as an important human respiratory pathogen causing both endemic and epidemic disease, including pneumonia, bronchitis, pharyngitis and sinusitis.[9,10] Up to 10% of community-acquired pneumonia cases in adults and up to 50% of pneumonia cases during epidemics are caused by this organism emphasizing the role of the respiratory system in this cross-infection. Although association of this organism with coronary heart disease, erythema nodosum and asthma has been demonstrated it is not clear how this organism is transferred from respiratory system to the circulation.[9,10]

It seems that macrophages which have helped to clear C. pneumoniae from the respiratory system under certain conditions become active carriers of the microbe. And when an infected macrophage travels down on a vessel wall, it may infect the cells lining the arterial surface. Under these conditions, the artery would attract more immune cells, which will deliver more bacteria to the site and cause more serious inflammation. This hypothesis with preliminary evidence supporting it nicely is depicted in Figure 1.

Researchers at Johns Hopkins and Louisiana State University have shown in test-tube experiments (in-vitro) that C. pneumoniae can indeed survive within macrophages and arterial cells.[28-31]

In these studies the ability of C. pneumoniae to infect cells that make up atherosclerotic lesions, including endothelial cells, smooth muscle cells, and cholesterol-loaded smooth muscle cells was examined. It was shown that C. pneumoniae can infect rabbit, bovine, and human smooth muscle cells, and cholesterol-loaded smooth muscle cells were more susceptible to C. pneumoniae infection.[28-31]

At the same time C. trachomatis inefficiently infected smooth muscle cells, demonstrating that this is not a characteristic of all members of the genus Chlamydia.[31]

It was concluded that C. pneumoniae has the capacity to infect one of the most important types of cells (smooth muscle) found within atherosclerotic lesions.

Recent animal studies reinforce evidence found in test-tubes during 1997 where different groups from Canada, USA and Finland have shown that C. pneumoniae invades arterial tissues.[28-31]

In these studies researchers infected a dozen rabbits through the nose and within seven weeks of contracting the organism, the majority of them developed arterial plaques. This plaque formation occurred despite the fact that rabbits do not normally get atherosclerosis, even when they are fed high-fat diets.

Even with this extensive information about the role of C. pneumoniae in heart disease, there will still be plenty of questions.

1. What is the mechanism of action?

2. Why are some people more susceptible than others?
3. Do fat and cholesterol make us sick by themselves, or only in combination with C. pneumoniae?
4. Could antibodies quickly rid the body of infection?
5. Would clearing the infection stop the disease process?

## A. Laboratory Gold Standards For Detection Of Chlamydia Pneumoniae

Culture, immunofluorescence, enzyme immunoassay and polymerase chain reaction (PCR) have been described in testing for C. pneumoniae.[32-35] By comparison of performances of different testing methods on sputum from 61 patients with respiratory disease it was concluded that:

PCR was 95% sensitive; 95% specific with positive predictive of 91% and negative predictive of 98%.

Enzyme immunoassay was 80% sensitive; 88% specific with positive or negative predictive values of 76% and 90% respectively. While culture technique was only 60% sensitive, 100% specific with positive predictive value of 100% and negative predictive value of 84%.

With nasopharynx and throat samples PCR has proven to be between 93-95% sensitive; 100% specific and with positive predictive value of 100% and negative predictive value of 100%. This makes PCR, especially nested PCR superior to the other available laboratory methodologies.[33] From this we conclude that in order to increase the sensitivity and specificity of clinical specimens for C. pneumoniae, the following combination of tests should be performed.

1. PCR which is the first-choice method for C. pneumoniae detection.
2. PCR results should be followed by southern blot using specific probe to C. pneumoniae.
3. Enzyme immunoassays for detection of IgG, IgM and IgA against specific peptide coding sequence for the major membrane protein of C. pneumoniae but not against C. trachomatis.

All these tests are done exclusively at Immunosciences Lab.

## B. Treatment of Chlamydia Pneumonia

Similar to Lymes disease and mycoplasma infection, and due to the intracellular nature of C. pneumoniae, long term antimicrobial treatment is needed. This extensive antimicrobial treatment is required for eradication of C. pneumoniae from macrophages and endothelial cells of infected arteries. Many researchers are now gearing up to test medications such as tetracycline; erythromycin; ofloxacin; clinafloxacin; ciprofloxacin; azithromycin; clindamycin; doxycycline and minocycline.[36-38]

In a preliminary study, researchers at St. George Hospital in London treated 46 survivors of heart attack with azithromycin. After six months, blood tests showed that the patients who were treated with this antibiotic were producing much less inflammatory proteins.[39] While in our laboratory we have the capability of measuring the inflammatory proteins such as fibrinogen and anti-oxidized LDL.[39-40] The real answer for diagnosis and treatment of C. pneumoniae, will be given only after measuring the bacterial load by quantitative PCR and by designing an accurate and sensitive antimicrobial sensitivity testing.

Unfortunately, many conventional treatments against Chlamydia fail. This failure causes significant rate of recurrence and morbidity. Therefore accurate and reproducible antimicrobial susceptibility test for Chlamydia have considerable clinical implications.

For this reason, we have developed a reverse transcriptase based method not only for detection and determination of Chlamydial load, but for reporting the antibiotic which this organism is most susceptible to.

Based on this PCR and its inhibition by the lowest concentration of specific antibiotic we found that: Doxycyline and tetracycline were the most active agents, followed by erythromycin and ciprofloxacin. Other antibiotics such as trimethoprim or sulphamethoxazole were ineffective against Chlamydia pneumoniae.

If based on antibiotic sensitivity assay described above, bacterial numbers will be decreased or eliminated from macrophages and infected tissues and if that effect will translate into improved symptomatologies and survival then, we can claim that cardiology is in for revolution.

For further information about:

1. The most sensitive method for detection of Chlamydia pneumoniae.
2. Southern blot confirmation of Chlamydia pneumoniae.
3. Detection of IgG, IgM and IgA antibodies to Chlamydia pneumoniae.
4. Measurement of Ig, IgM and IgA immune complexes specific to Chlamydia pneumoniae.
5. Determination of IgG, IgM and IgA against oxidized LDL and other inflammatory proteins.

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Reference