

CURRENT OPINION

Infection with *Chlamydia pneumoniae* as a cause of coronary heart disease: the hypothesis is still untested[#]

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One-Sentence Summary: Review of the possible role of *Chlamydia pneumoniae* infection in the pathogenesis of heart disease.

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We are convinced that *Chlamydia pneumoniae* research has been unfavorably affected by the negative results of antibiotic treatment trials for the secondary prevention of late-stage coronary heart disease (CHD) (O'Connor et al., 2003; Cannon et al., 2005; Grayston et al., 2005). There is a widespread belief that the clinical trials showed that *C. pneumoniae* has no role in atherosclerotic disease. This flawed causal inference from these trials has contributed to slowing much-needed research on *C. pneumoniae*. It has also resulted in a nearly complete loss of momentum for research on the infection-based response to injury hypothesis as a key factor in the initiation and progression of CHD.

Box 1

Chlamydia pneumoniae had been considered a possible cause of atherosclerosis. That antibiotics failed to prevent secondary coronary events in patients with established coronary artery disease has been erroneously interpreted as ruling out a causative role for *C. pneumoniae*. This misinterpretation has had a chilling effect on *C. pneumoniae* research.

Our concern has been confirmed by the sharp drop in published reports (PubMed citations) on *C. pneumoniae* since 2005. From 1999 to 2005, approximately 375 papers were published each year. There has been a 62% drop from that number with 143 being listed for 2013.

The antibiotic treatment trials were not etiologic studies. No inference regarding the role of *C. pneumoniae* in the cause of atherosclerosis can properly be made from the trials. A prior publication stated that the study design for these trials precluded proving or disproving a role for *C. pneumoniae* in the initiation or progression of atherosclerosis, and predicted an over-reaction to either negative or positive results (Grayson 2000).

All subjects of these trials had established coronary artery disease with mostly advanced disease. Most had had a myocardial infarction (MI). The trials studied whether antibiotics could prevent secondary coronary events in patients who previously had had a coronary event. A coronary event was defined as an MI, a specified angina episode, surgical intervention or death.

While it was disappointing, it was not surprising that antibiotics given late in the course of atherosclerotic arterial disease did not prevent additional coronary events. The pathogenesis of MI has differences from the pathogenesis of atherosclerosis. In the animal model, antibiotic treatment was effective only when given early after *C. pneumoniae* inoculation. The animal model treatment studies were published after most of the clinical trials had been initiated.

Authors' discussion of the lack of progress in *C. pneumoniae* research

The misinterpretation of the significance of the clinical trial results was already becoming known when the authors first began discussions that eventually led to this manuscript.

These discussions occurred at a remote mountainous ranch in Wyoming in 2007. The purpose of this 'think tank' was to discuss the state of *C. pneumoniae* research and to recommend areas for emphasis in future investigations. The stimulus for the retreat was our concern with the lack of progress in *C. pneumoniae* research.

A wide variety of research needing attention was discussed. During the second day of the discussions, the group was coalescing around the idea that while there were many individual technical and conceptual problems in *C. pneumoniae* research, the most important was the potential role of *C. pneumoniae* infection in atherosclerotic diseases.

The decisions to select this issue as the one for further study and to develop possible approaches for future research were influenced by the enormous importance of atherosclerotic diseases to human health. Atherosclerotic CHD is now the no. 1 killer throughout the industrialized world.

Two major topics

This manuscript will present two major topics: first, a description of the evidence for a potential etiologic association of *C. pneumoniae* and atherosclerosis, and second, a proposed method that could provide data for an etiologic association.

Infection has been proposed as a causal factor in heart disease for more than 100 years. In the last two decades, there has been increasing research on the role of infection in atherosclerotic cardiovascular disease. The accumulated data associating *C. pneumoniae* with atherosclerosis are particularly compelling.

C. pneumoniae in atherosclerosis

C. pneumoniae is unique among several microbes that have been associated with arterial disease in having been demonstrated with frequency in atherosclerotic lesions but not in normal arterial tissue (Kuo et al., 1995). The organism was found not only in coronary arteries but also in carotid (Jackson et al., 1997), aortic (Kuo et al., 1993), femoral and popliteal arteries (Kuo et al., 1997). These findings have been confirmed by a number of experienced investigative teams using multiple laboratory techniques, including PCR, microscopy with immunocytochemical stain, in situ DNA hybridization and isolation of the organism (Campbell and Kuo 2004; Watson and Alp 2008). In atheromas, *C. pneumoniae* is found within smooth muscle cells, macrophages and endothelial-derived foam cells (Kuo et al., 1993; Kuo and Campbell 2000).

In addition to the human studies, animal studies have contributed to the evidence for *C. pneumoniae* as an infectious cause of CHD. *Chlamydia pneumoniae* pulmonary infection has been shown to accelerate atherosclerotic disease in mice and rabbits prone to develop the disease due to genetic manipulation or high-fat diets (Muhlestein et al., 1998; Hu, Pierce and Zhong 1999; Moazed et al., 1999). In rabbits fed a regular diet, *C. pneumoniae* infection resulted in atherosclerotic changes (Fong et al., 1999). In contrast, although repeated infection did not induce atherosclerosis in normolipidemic mice, inflammatory changes were observed in the heart and aorta (Blessing et al., 2000). If a high-fat diet was initiated concurrently with infection in C57BL/6 mice, atherosclerotic lesion progress was accelerated (Blessing et al., 2002a). However, if infections preceded administration of high-fat diet, no augmentation of lesion development was observed. Taken together, these results suggest that in the mouse model *C. pneumoniae* infection is a co-risk factor with hyperlipidemia.

In human atherosclerotic lesions, although the organism has been cultured only a few times, the organism is frequently detected in mature atherosclerotic lesions by other methods, suggesting persistent infection. Similarly, following repeated pulmonary infection in mice, the organisms can be cultured from the aorta for 1–2 weeks post-infection, but can be detected by other methods in the aorta for 20 weeks post-infection, the end-point of the experiment (Moazed et al., 1997).

In rabbit models of *C. pneumoniae* accelerated atherosclerosis, azithromycin prevented the accelerated intimal thickening. However, timing of antibiotic treatment was critical in blocking *C. pneumoniae* accelerated atherosclerosis. Specifically, initiation of treatment with either clarithromycin or azithromycin within a week after the first of three inoculations with *C. pneumoniae* was efficacious, while delayed treatment initiated 6 weeks after the first inoculation was not (Fong 2000). Studies done in *apoE* knockout mice using different treatment regimens of azithromycin (a dose after each of two inoculations or a 6-week course initiated after the third inoculation) did not demonstrate any reduction in *C. pneumoniae* accelerated atherosclerosis (Rothstein et al., 2001; Blessing et al., 2005). Importantly, following antibiotic treatment in both rabbit and mouse models, *C. pneumoniae* DNA or antigen was detected in the aorta, suggesting that infection was refractory to treatment (Muhlestein et al., 1998; Rothstein et al., 2001; Fong et al., 2002).

Other biological effects consistent with a role of *C. pneumoniae* in atherosclerotic processes have been demonstrated in animal models including increased T-cell influx into the atherosclerotic lesion and earlier formation of complex lesions (Ezzahiri et al., 2002), enhanced endothelial dysfunction (Liuba et al., 2000, 2003), apoptosis of endothelial cells and degenerative changes

associated with necrosis (Birck et al., 2013), and plaque destabilization as suggested by increased production of matrix metalloproteinases and reduced area of the fibrous cap (Ezzahiri et al., 2003) as well as increased intra-plaque hemorrhage in older mice (Campbell et al., 2010).

The human and animal studies have been used to demonstrate the role of *C. pneumoniae* infection in atherosclerosis. The question of how *C. pneumoniae* infection exacerbates atherosclerosis has been addressed in cell culture systems. In the presence of LDL, *C. pneumoniae* induces foam cell formation and simulates LDL oxidation, through chlamydial LPS and Hsp60, respectively (Kalayoglu and Byrne 1998; Kalayoglu et al., 1999a,b). Scavenger receptors mediate oxidized LDL (oxLDL) uptake (Ross 1993).

For endothelial cells, the lectin-like oxLDL receptor (LOX-1) is the major receptor for uptake of oxLDL (Sawamura et al., 1997; Kume et al., 1998). This scavenger receptor is also found on macrophages and smooth muscle cells (Moriwaki et al., 1998; Aoyama et al., 2000). Expression of LOX-1 is increased in hyperlipidemia and atherosclerotic lesions and activation of LOX-1 results in the up-regulation of pro-atherogenic factors (Kataoka et al., 1999; Chen et al., 2000; Li and Mehta 2000; Li et al., 2003; Zhu et al., 2005). *Chlamydia pneumoniae* has been shown to bind to the LOX-1 receptor, up-regulate LOX-1 expression, induce the expression of adhesion molecules and matrix metalloproteinases through LOX-1 activation, and promote uptake of oxLDL (Yoshida et al., 2006; Campbell et al., 2012, 2013). *Chlamydia pneumoniae* infection of macrophages inhibits the expression of the cholesterol transporters ABCA1 and ABCG1 which play critical roles in cholesterol efflux and homeostasis (Liu et al., 2010; Korhonen et al., 2013; Zhao et al., 2014).

Chlamydia pneumoniae infection of vascular cells also induces the expression of pro-inflammatory cytokines, chemokines and growth factors, all of which could contribute to the chronic inflammatory processes of atherosclerosis (Kaukoranta-Tolvanen et al., 1996; Hu, Pierce and Zhong 1999; Gaydos 2000; Kothe et al., 2000; Netea et al., 2000, 2002; Summersgill et al., 2000; Coombes and Mahony 2001; Blessing et al., 2002b; Mamata et al., 2007; Eitel et al., 2012).

The findings of *C. pneumoniae* in atherosclerotic lesions provided the impetus for a number of investigators to attempt treatment of CHD with antibiotics known to be effective against *C. pneumoniae*. Results from three large randomized double blind studies of the effect of antibiotic treatment for secondary prevention of coronary events have been reported (O'Connor et al., 2003; Cannon et al., 2005; Grayston et al., 2005). Two of these studies had prolonged antibiotic treatment for one year (Cannon et al., 2005; Grayston et al., 2005). There were no differences in outcome between the groups given antibiotics or placebo. The overall results from a number of smaller trials were similarly negative (Andraws, Berger and Brown 2005). The significance of the failure of antibiotics to reduce coronary events in patients with established CHD has been discussed above.

Childhood atherosclerosis and *C. pneumoniae*

It is now well established that both atherosclerosis and *C. pneumoniae* infection are first seen in early childhood and that the prevalence of both increase with age.

A variety of studies in different populations using different techniques have shown that atherosclerosis begins in childhood. In autopsy studies in Japanese children, evidence of early atherosclerosis, fatty streaks, was found in 29% of aortas in those <1 year old and in 3.1% of coronary arteries of children

1–9 years old (Tanaka et al., 1988). In a US autopsy study of coronary arteries, the prevalence of fatty streaks in the coronary arteries increased with age from 50% at 2–15 years of age to 85% at 21–39 years of age. Raised fibrous-plaque lesions were seen in 8% of children 2–15 years of age and in 69% of adults 26–39 years of age (Berenson et al., 1998). Another study showed lipid-laden macrophages in the intima of the aorta and coronary arteries of young American children killed in motor accidents, with over 50% aged 10–14 years having some evidence of early atherosclerosis (Stary 1989). In the Pathobiological Determinants of Atherosclerosis in Youth study (McGill et al., 2000), in those 15–19 years old at time of death, raised fatty streaks were found in 20% of aortas and 10% of right coronary arteries. The prevalence increased to 40% of aortas and 30% of right coronary arteries showing the lesions by age 30–34.

Box 2

Studies on the etiology of atherosclerosis must begin early in life:

1. Atherosclerotic lesions have been found in very young children and the prevalence increases with age.
2. *Chlamydia pneumoniae* infection first appears in early childhood and accelerates with school attendance.
3. In animals, *C. pneumoniae* pulmonary infection accelerates development of atherosclerotic disease.

Utilizing an intravascular ultrasound technique, it was found that 17% of otherwise healthy heart donors <20 years of age, 37% of those aged 20–29 years, 60% of those aged 30–39 years, 71% of those aged 40–49 years and 85% of those ≥50 years of age had evidence of coronary intimal thickening (Tuzcu et al., 2001).

Seroepidemiologic studies with the microimmunofluorescence (MIF) test have provided information on *C. pneumoniae* infections in populations. Using several different serum banks, data on the prevalence and incidence of *C. pneumoniae* infections at different ages have been obtained (Aldous et al., 1992; Grayston 1994). The age-specific prevalence of *C. pneumoniae*-specific IgG antibody has shown a similar curve in several countries. Fig. 1 shows age prevalence in over 1000 Seattle residents below 20 years of age. The greatest increase in prevalence is seen from 5 to 15 years of age. While the prevalence rate is low in children under 5, many of the mild infections in these very young children may not produce long-lasting IgG antibody. This

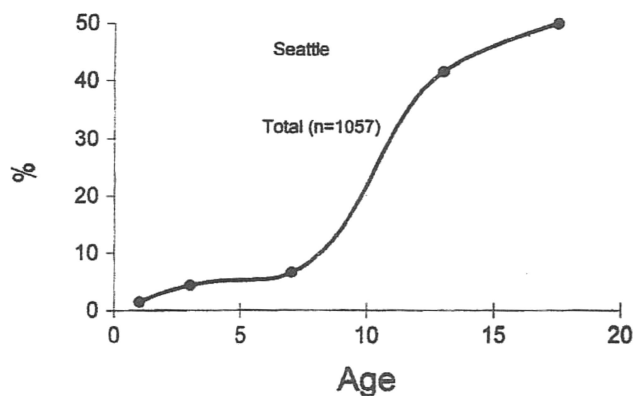


Figure 1. Prevalence of *C. pneumoniae* MIF IgG antibody by age in Seattle children (Grayston 1994).

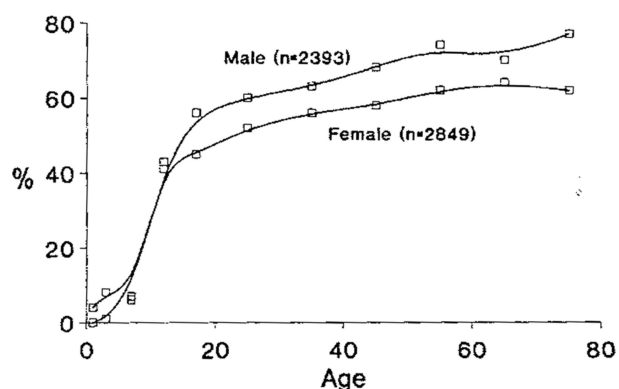


Figure 2. Prevalence of MIF antibody to *C. pneumoniae* (TWAR) by age in among 5242 persons in Seattle. IgG titers ranged from 8 to 256 (Grayston 1992).

phenomenon has been demonstrated in very young children infected in the eye with *C. trachomatis*. Often two or three reinfections occur before antibody persists (Grayston et al., 1985).

Sera from a family study in Seattle from 1963 to 1979 (Aldous et al., 1992) were used to determine incidence rates of *C. pneumoniae* infection based on 4-fold antibody titer rises. High annual rates of antibody rises were seen in the 5–9 year age group (9.2%) and the 10–14 year group (6.2%). These rates are consistent with the increase in prevalence rates from 5–14 years. The available clinical data suggest that many of the antibody conversions occurred in children who were asymptomatic. The symptomatic children usually had cough, a hallmark of *C. pneumoniae* disease. These incidence rates were confirmed by a study of sera collected longitudinally from children in Sweden. The reported annual MIF antibody conversion rates were 8.0% in 8 to 12 year olds and 5.9% in 12 to 16 year olds (Haidl, Sveger and Persson 1994).

Chlamydia pneumoniae antibody prevalence rates continue to rise throughout adult life reaching 70–80% in the elderly. The rates are approximately the same in both sexes until age 15. Thereafter, the rates are higher in males, Fig. 2. The *C. pneumoniae* gender prevalence is different from other organisms causing respiratory infections where the prevalence rates are higher in women, presumably because they are in closer contact with children who are often the source of family infection. There is no known reason for *C. pneumoniae* to be more common in males but it is intriguing that atherosclerotic diseases are more common in males.

How to demonstrate *C. pneumoniae* etiology of CHD

We know of no simple ways to prove *C. pneumoniae* etiology of CHD and have proposed an expensive lengthy observational study supported by laboratory studies. Analogies could be the Framingham Heart Study (Dawber, Kannel and Lyell 1963) and the virus watch studies (Fox 1974). The plan calls for a large-scale natural history study to evaluate a causal link between *C. pneumoniae* and atherogenesis. This ambitious project is made feasible by recent advances in diagnostics for both early atherosclerosis and *C. pneumoniae* infection.

Family studies

The use of longitudinal evaluation of families to study the epidemiology of chronic infections, originally championed by Wade Hampton Frost (1880–1938), the first professor of Epidemiology in the United States at Johns Hopkins University. Frost's interests spanned the use of family-based data sets to study the epidemiology of both acute (e.g. influenza) and chronic (e.g. tuberculosis) infections, mainly in the 1920s and 1930s (Daniel 2009). Frost recognized that determinants of risk for individuals exposed to agents that cause chronic infections, as summarized by Fox (Fox 1974), are similar to those for pathogens that cause acute infections. The same three basic facts are required. These are (i) onset data for the first case (index case), (ii) roster of family members present and (iii) dates of successive cases. However, accurate assessment was found to be much more difficult for chronic infections since risk is not concentrated in the relatively brief period after exposure, but rather causal morbidity and mortality may occur at any subsequent time in the life of the infected individual. Frost found that two major values of family-based epidemiologic studies include developing reliable estimates for the risk of transmission of specific infectious agents, and the capacity to follow the natural history of specific pathogens. The latter proved to be especially valuable for chronic infections where most exposed individuals remained asymptomatic (Fox 1974). The so-called 'virus watch' method that was adopted by family-based epidemiologists who followed Frost generally assumes that most infected individuals will be subclinical and therefore the emphasis of sampling is on the occurrence of infection rather than the development of disease, especially acute disease.

The prototype for this sort of study is the polio virus watch investigation carried out by Fox and colleagues in Louisiana from 1953 to 1957, where it was established that subclinical infection far exceeded progression to paralytic disease (Fox et al., 1957; Gelfand et al., 1957). The polio virus watch study, which involved multiple sample collections for virus isolation in family members, antibody seroconversion measurements and verification of disease status in each household, was reproduced in a number of other family-based epidemiologic studies to gain insights into influenza, adenovirus, mycoplasma and tuberculosis infections (Fox 1974). The impact of the virus watch programs was extremely helpful in defining epidemiologic patterns for a number of acute and chronic viral and bacterial diseases. Similar, albeit far earlier, epidemiologic surveys were used over the course of 30–40 years to establish links between streptococcal pharyngitis, rheumatic fever and rheumatic heart disease, despite great initial resistance to seriously considering an infectious agent contributing to this very large public health problem in children (Benedek 2006), a condition now that is effectively avoided by prompt treatment of streptococcal pharyngitis (Guzmann-Cottrill et al., 2004).

The natural history of atherosclerotic cardiovascular disease has its genesis early in life. Carotid artery intima-media thickness in children and young adults, coupled with other risk factors (e.g. LDL cholesterol, elevated body mass index), predict atherosclerosis in adulthood (Juonala et al., 2010). Given the known involvement of infectious agents in chronic inflammatory events, and the existing associations between *C. pneumoniae* respiratory infections and atherosclerotic diseases, it should be possible to utilize the family-based epidemiologic methodologies to add to our knowledge on the role of *C. pneumoniae* infections in cardiovascular disease.

Non-invasive measures of atherosclerosis

Basic cardiovascular research has led to a greater understanding of pathological processes underlying atherosclerosis, from initiation to progression of arterial plaques. Imaging techniques have also advanced beyond their use in merely defining anatomic features, and may now permit research efforts to probe cellular interactions, metabolic pathways and functional outcomes in the causal pathway of atherosclerosis and plaque rupture.

The thickness of the carotid artery intima measured by high-resolution ultrasonography may aid in detecting early vascular changes of atherosclerosis (Osika et al., 2007). The use of computed tomography (CT) has limited use in children because of the radiation exposure. Magnetic resonance imaging (MRI) is increasingly used to assess early atherosclerosis, since it allows characterization of cardiovascular structure, function and blood flow without exposure to ionizing radiation. It has been used to measure atheromatous plaques and characterize structure in the peripheral circulation. Both CT and MRI are currently being combined with positron emission tomography and fluorescent imaging to evaluate plaque biology in more detail (Sosnovik, Nahrendorf and Weissleder 2007). Arterial stiffness (atherosclerosis) can be assessed noninvasively by measuring a parameter known as the pulse wave velocity (PWV) between two major arteries located in the upper body (i.e. carotid or brachial artery) and the lower body (i.e. femoral or ankle). PWV reflects the time needed for the pulse wave to travel a given distance along the blood vessel. Stiffer arteries produce a higher PWV (Alpert and Collins 2007). PWV may have an advantage compared to other non-invasive techniques due to its simplicity of use and shorter time required for a measurement. A recent review (Camici et al., 2012) summarized the published literature reporting on advanced imaging techniques that have great potential for broad application in clinical practice. Studies will be needed to further prospectively evaluate these techniques. However, one can imagine that soon high-resolution methods will be compatible for use in longitudinal studies of atherosclerosis and underlying etiology.

Diagnosing *C. pneumoniae* infection

Although efforts have been made to standardize the detection of *C. pneumoniae* infection (Dowell et al., 2001), there is still lack of consensus on methods for differentiating acute versus chronic/persistent infection with *C. pneumoniae*. Nevertheless, past research efforts have provided sufficient information to choose and optimize diagnostic approaches suitable for monitoring *C. pneumoniae* infection for a family surveillance study. A list of approaches that can be considered for diagnosing *C. pneumoniae* airway infection is provided below.

- (i) PCR-based detection of *C. pneumoniae*-unique DNA sequences: this approach is both sensitive and specific (Al-Marzooq et al., 2011; Cho et al., 2012; Diaz and Winchell 2012). It can also be quantitative, which will allow infection burden to be correlated with clinical phenotypes.
- (ii) Live *C. pneumoniae* organism isolation: although it is highly specific and can also provide materials for further characterization studies, the success rate is low lacking the required sensitivity as a routine screening tool (She et al., 2010). Improvement of isolation methods is needed.
- (iii) Immunolabeling-based detection of *C. pneumoniae* antigen: besides the choice of target antigens and selection of an-

tibodies, the assay platforms can also significantly affect both the detection specificity and sensitivity. Until they are improved, these assays will likely generate highly variable results and not be suitable for the proposed surveillance study.

- (iv) Detection of *C. pneumoniae*-induced host responses including cytokines, antibodies and T cells: although *C. pneumoniae* is known to induce inflammatory cytokines via activating innate immunity receptor-mediated pathways, detection of these cytokines cannot be used for diagnosing *C. pneumoniae* infection due to the lack of specificity of the innate immunity receptors for recognizing *C. pneumoniae*. Only the host immune responses mediated by *C. pneumoniae* epitope-specific immune receptors can be used for indicating *C. pneumoniae* infection. However, due to the long half-life of immune molecules and memory lymphocytes, the immune response detection-based assays can only be used to indicate whether the subjects have ever been infected with *C. pneumoniae*. T-cell responses are more difficult to measure and also require more blood samples. Thus, T-cell responses are not suitable for routine screening for *C. pneumoniae* infection. However, *C. pneumoniae* epitope-specific T-cell responses can be measured for investigating the roles of T-cell responses in *C. pneumoniae* pathogenesis.
- (v) Next-generation sequencing (NGS) (van Dijk et al., 2014) can be used for identifying co-infection agents and profiling microbiome in the airway (Goodrich et al., 2014). NGS can be used to target either the variable regions of 16s RNA genes (Langille et al., 2013) or cover the entire microbial genomes (Song, Jarvie and Hattori 2013) for both identifying microbial species and quantitating the relative amounts of each species in a given sample.

For screening participating families: nasopharyngeal swabs and peripheral blood mononuclear cells (PBMC) can be used for monitoring acute *C. pneumoniae* infection by detecting *C. pneumoniae* DNA and for profiling the microbiota and co-infection agents by using NGS. Since the microbiota and co-infection agents in the airway likely affect the pathogenicity of *C. pneumoniae*, it is important to simultaneously monitor the microbial species co-existing in the nasopharyngeal and peripheral blood cells. At the same time, the plasma samples left from the above preparation of PBMC will be used for determining *C. pneumoniae* exposure status. Both the overall titers and antigen specificities of *C. pneumoniae*-specific antibodies will be determined. The latter can be carried out as described for mapping *C. trachomatis* antibody specificities (Wang et al., 2010). These measurements together will establish a baseline for each study subject family.

During follow-up, the same samples will be collected periodically (as determined for observing clinical phenotypes) for monitoring both *C. pneumoniae* infection and the microbiota and host antibody responses.

These laboratory-measured parameters will be correlated with clinical responses observed in the same study subjects/families.

- (i) Correlation of *C. pneumoniae* infection rate and burden with clinical diseases will allow identification of disease with *C. pneumoniae* infection.
- (ii) Correlation of airway microbiota profiles and co-infection status with clinical diseases will allow us to map airway microbial species associated with clinical diseases or lack of diseases.

- (iii) Correlation of anti-*C. pneumoniae* antibody titers and antigen-specificity profiles with clinical diseases will allow us to identify both pathogenic and protective antigens as has been done for *C. trachomatis*-infected women (Budrys *et al.*, 2012).
- (iv) Correlation between the above three parameters will allow investigation into the impact of the airway microbiota and co-infection on *C. pneumoniae* infection and pathogenicity, and on host responses to *C. pneumoniae* infection.

CONCLUSIONS

A family study could be designed that would provide information about atherosclerotic disease status among family members and the prevalence of *C. pneumoniae* in nasopharyngeal swabs and PBMC, as well as changing titers of host humoral and cellular responses to *C. pneumoniae* antigens. Advances in *Chlamydia* proteomics and genomics offer the promise of improved diagnostic and immunologic tests to better define infection status (primary, secondary, persistent) (Bunk *et al.*, 2008). Clearly, new technologies involving highly sensitive and specific sequencing methods may be brought to bear on differential gene expression patterns associated with ongoing productive infection and *C. pneumoniae* persistence. These technologies associated with advances in vascular imaging may further help define those who suffer from infection-provoked vascular disease. Given the remarkable advances in non-invasive vascular imaging technologies, it would also be possible to obtain information on the initiation and progression of atherosclerotic lesions in large- and medium-sized arteries. Simultaneous longitudinal data on vessel health and infection status would provide insight into a possible etiologic association (Bunk *et al.*, 2008). The plan would encourage evaluation of many other potential etiologic factors (e.g. obesity, hypercholesterolemia and other microbes).

For *C. pneumoniae* research, a number of applied and basic benefits would be derived from such a program. These include the opportunity to develop and implement new diagnostic tests, the creation of much needed collections of *C. pneumoniae* isolates for genomic studies, improved understanding of the relationship between immune responses and disease pathogenesis, mapping *C. pneumoniae* antigens associated with protective immunity vs. pathological responses, and identification of genetic determinants associated with infection susceptibility and cardiovascular health.

The recent recommendations of the American Academy of Pediatrics to test blood cholesterol and in some circumstances to undertake cholesterol lowering treatment of young children emphasize the early age for prevention of heart disease (Volanen *et al.*, 2006). The proposed family study could include in its design studies that would add scientific rigor to the pediatric recommendations.

CHD is the leading cause of death in the industrialized world. Current patient management including life-style changes has reduced CHD deaths and improved quality of life for millions, but there is no cure for the disease. It is critical that the cause(s) of early atherosclerotic changes be determined, to provide an opportunity for prevention or cure. Both atherosclerosis and evidence of prior *C. pneumoniae* infection are found in most adults. If *C. pneumoniae* infection or other microbes are found to be closely associated with the initiation and development of CHD, a targeted antibiotic treatment or preferably a preventive vaccine could be tested. Given the magnitude of the problem, research to define the role of infection in heart disease should rightfully be a

top-tier national health priority. Studies such as a Family Heart Watch Program, despite the high cost, should be undertaken.

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