Chlamydia pneumoniae and Atherosclerotic Risk in Populations: The Role of Seroepidemiology

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While seroepidemiologic studies first suggested a possible association of prior infection with Chlamydia pneumoniae and atherosclerotic risk, the contribution of seroepidemiologic studies of C. pneumoniae and atherosclerotic risk remains a source of controversy, in part because the reported findings appear inconsistent [1–20]. In general, cross-sectional studies of C. pneumoniae and atherosclerotic risk suggest an association, but recent reports from several prospective studies failed to demonstrate associations between the presence of IgG antibodies to C. pneumoniae and incident myocardial infarction. Evidence from other paradigms—pathologic, animal experimental, and molecular studies—supports a possible etiologic role for C. pneumoniae in atherothrombotic disease, raising questions about the contribution of seroepidemiologic studies. This review summarizes the major findings from seroepidemiologic studies in the context of other research paradigms, explores alternative explanations for the inconsistent findings, and suggests a further role for seroepidemiologic studies of C. pneumoniae and atherothrombotic risk.

While seroepidemiologic studies first suggested a possible association between prior infection with Chlamydia pneumoniae and atherosclerotic risk, the contribution of seroepidemiologic studies of C. pneumoniae and atherosclerotic risk remains a source of controversy, in part because the reported findings appear inconsistent [1–20]. In 1988, Saiku et al. [1] reported a new association of IgG antibody to C. pneumoniae and coronary heart disease (CHD) among young Finnish men. Subsequent seroepidemiologic studies suggested an association of IgG antibody to C. pneumoniae with both coronary and carotid atherosclerosis [2–4]. These studies were followed by mounting evidence from pathologic, animal experimental, and molecular studies that supported a possible etiologic role of infection with C. pneumoniae in atherosclerosis [5–16]. However, recent reports from several prospective studies have failed to demonstrate an association between the presence of antibody to C. pneumoniae and incident myocardial infarction (MI) [17–20].

Infection with C. pneumoniae is a common cause of pneumonia, occurs early in life, has worldwide distribution, and can persist, possibly resulting in chronic infection [21]. In addition, as with other Chlamydia species, reinfection with C. pneumoniae also occurs. Microimmunofluorescence (MIF) is used to measure antibody (IgG, IgA, and IgM) to C. pneumoniae and is both a sensitive and specific technique for the detection of prior infection with C. pneumoniae [22]. In the setting of pneumonia, C. pneumoniae IgG titers typically increase to >1:512 or 1:1024 [22]. However, it is unclear whether the presence or titer of C. pneumoniae IgG or IgA antibody by MIF reflects persistent, chronic active, or reinfection.

The development of coronary atherosclerosis and acute MI is now thought to involve a series of steps: initiation, progression, plaque formation, plaque rupture or erosions, and thrombosis [23–25]. While the early lesions of atherosclerosis, raised fatty streaks, occur worldwide, progression of fatty streaks to atherosclerotic plaque occurs most commonly in Western societies characterized by a highly saturated fat diet and elevated serum cholesterol levels [23, 24]. Other factors may also contribute to the occurrence of plaque rupture or erosions and the thrombosis of a coronary artery that results in acute MI. If infection with C. pneumoniae was related only to the early stages of atherothrombotic disease, the inconsistent seroepidemiologic findings might result from differences in the outcomes examined in prior studies.

Given the known limitations of the serologic measures of prior infection and the generally positive findings from other research paradigms summarized elsewhere in this supplement, some have questioned the contribution of seroepidemiologic studies to the association of C. pneumoniae and atherosclerotic risk. Here we briefly review the major findings from seroepidemiologic studies in the context of other research paradigms, identify limitations of prior seroepidemiologic research, and suggest alternative explanations for the apparently inconsistent findings from prior seroepidemiologic studies. We then suggest a role for seroepidemiologic studies related to C. pneumoniae and human atherosclerotic risk.
Findings from Other Research Paradigms

Since seroepidemiologic studies need to be considered in the context of other research paradigms, we briefly review prior findings below. Numerous studies have demonstrated the presence of *C. pneumoniae* in coronary artery, carotid artery, and abdominal aorta atheroma by immunocytochemistry (ICC), polymerase chain reaction, and electron microscopy [5–9]. In contrast, *C. pneumoniae* antigens are not detectable in normal arterial walls or nonatherosclerotic artery segments of persons with atherosclerosis. *C. pneumoniae* cultures from atheromatous plaque show that viable organisms are present in vascular tissue [10]. *C. pneumoniae* infects the cells involved in atherosclerosis—endothelial cells, smooth muscle cells, and macrophages—providing evidence for a mechanism by which the organism can infect the vessel wall [11]. The organism alters cell function in ways that can promote atherothrombotic disease [12]. For example, infection with *C. pneumoniae* promotes the formation of foam cells and the presence of adhesive molecules on the surface of endothelial cells. In general, the findings from both pathologic and molecular studies suggest the possibility that atherothrombotic disease could result, at least in part from persistent, chronic active, or recent infection with *C. pneumoniae*, consistent with the response-to-injury hypothesis of atherosclerosis [23].

In a variety of animal experimental models, repeated infection with *C. pneumoniae* through the respiratory tract has induced early lesions or accelerated progression of atherosclerosis in the aorta [13–16]. Of note, an isolated episode of infection typically is not adequate to induce atherosclerosis in various animal models. Furthermore, animal experimental studies consistently demonstrate a potential interaction between infection with *C. pneumoniae* and elevated serum lipids resulting from dietary and/or genetic factors [13–15]. In the presence of elevated serum cholesterol, repeated respiratory tract infection with *C. pneumoniae* results in the early changes of atherosclerosis and in the absence of elevated serum cholesterol; repeated infection does not induce the early changes of atherosclerosis. Of particular importance, animal experimental data also demonstrate that antibiotic treatment can prevent or reduce atherosclerotic changes in the aorta of animals repeatedly infected by *C. pneumoniae* [13].

Findings from Seroepidemiology

Seroepidemiologic studies have examined the associations of prior infection with *C. pneumoniae* with different stages of the atherothrombotic process in defined human populations [1–4, 17–20]. In the studies, efforts were made to minimize the effect of other factors that might potentially confound the association of prior infection with atherothrombotic disease. Analyses were also conducted to identify factors that alter or influence the association between infection and atherothrombotic disease, since in some persons infection could be associated with an increased risk of atherothrombotic disease while in others infection may be associated with little or no increased risk. In addition, seroepidemiologic studies can explore potential mechanisms through which infection might influence atherosclerotic risk in populations, such as through effects on inflammation, as reflected by circulating inflammatory markers (e.g., serum fibrinogen, C-reactive protein, and interleukin-6) or the progression of subclinical atherosclerotic disease as reflected by carotid ultrasound evidence of subclinical atherosclerosis.

Seroepidemiology of Atherosclerosis

Several studies examined the association of antibody to *C. pneumoniae* and coronary and carotid atherosclerosis as determined by coronary angiography and carotid ultrasonography [2–4]. In a population-based angiography case-control study conducted in Seattle, Thom and colleagues [2, 3] demonstrated that IgG antibody is associated with an increased risk of coronary atherosclerosis; however, the increase in risk occurred only in current or former smokers. The presence of antibody also was associated with an increased ultrasonographically measured carotid artery wall intimal-medial thickness, a marker of carotid atherosclerosis, in the Atherosclerotic Risk in Communities (ARIC) Study [4].

Seroepidemiology of incident MI and CHD death. While the initial cross-sectional studies, in which both IgG antibody levels and coronary atherosclerosis were determined at the same time, were positive, most but not all prospective studies of infection with *C. pneumoniae* and incident MI and CHD death have not demonstrated an association [17–20], either overall or within high-risk subgroups [17–20]. The initial report from the Helsinki Heart Study in hyperlipidemic men was interpreted as suggestive of an association. Only the presence of both IgA antibody and lipopolysaccharide immune complexes 3 months prior to the event was associated with an increased risk of MI [17]. In contrast, IgG measurements and IgA measurements made earlier were not associated with the risk of MI. Recently, a case-cohort analysis from the ARIC study and nested case-control studies from the Physicians Health Study, the Nurses Health Study, and the Caerphilly Study failed to demonstrate an association between the presence of IgG antibody to *C. pneumoniae* with incident MI [17–20]. However, in the ARIC study, there was a modest association among nonsmokers, and high-titer IgG antibody (1:512 and 1:1024) was more prevalent among cases of incident CHD than among noncases. The other prospective studies did not report findings among persons with these high titers, possibly because a relation with high-titer IgG was not an a priori hypothesis.

Limitations of seroepidemiologic studies. Several limitations need to be considered when interpreting the findings from prior seroepidemiologic studies. With the exception of the nested case-control analysis of the Helsinki Heart Study, prior studies used a single measurement of IgG antibody to assess “expo-
sure” to \textit{C. pneumoniae}. As noted earlier, while the presence of IgG antibody likely reflects the presence of prior infection, it may not be a valid and reliable measure of persistent, chronic active, or reinfection. In general, the focus of measurement has been on classifying persons as seropositive or not, in part because the meaning of a single IgG titer to \textit{C. pneumoniae} has been unclear. The interpretation of studies reporting on risks in seropositive and seronegative subjects is further complicated by the fact that the definition of seropositivity has varied in different studies from titers $\geq 1:8$ or $1:16$ to titers $\geq 1:64$, $1:128$, or $1:256$. Few studies have examined the associations of various stages of atherothrombotic disease with the full range of titers (e.g., $\geq 1:512$ or $1:1024$) [18]. Since \textit{C. pneumoniae} titers can persist, it is possible that titers below 1:512 may reflect prior infection and not persistent, chronic active, or reinfection. As noted, paired sera obtained from pneumoniae cases has suggested that a titer of 1:512 or 1:1024 likely reflects recent infection. Furthermore, a small study failed to show a relation between low-to-moderate IgG titers and the presence of \textit{C. pneumoniae} antigens detected by ICC in carotid artery plaques [26]. Whether high-titer IgG or IgA antibodies are better measures of persistent, chronic active, or reinfection remains a source of controversy.

Prior studies have focused on antibody response to \textit{C. pneumoniae} because it has not been possible to assess the associations of circulating \textit{C. pneumoniae} antigens in monocytes with atherothrombotic disease. It is possible that the use of antibody as a marker of prior infection is not adequate to identify those at atherosclerotic risk from infection with \textit{C. pneumoniae}, particularly if persistent, chronic active, or reinfection is the exposure associated with an increased risk. For other infections that cause chronic disease, such as hepatitis B virus and hepatocellular carcinoma, a specific marker of chronic or persistent infection was needed to identify an association. A similar approach may be needed to identify the true association between infection with \textit{C. pneumoniae} and atherosclerotic risk. At present, it is unclear whether the measurement of \textit{C. pneumoniae} antigens in circulating monocytes will provide a better marker of persistent, chronic active, or reinfection than available serology.

The apparent inconsistency of prospective and cross-sectional studies of the associations of prior infection with coronary and carotid atherosclerosis has raised concerns regarding the temporal relation between infection and atherosclerotic risk. For example, does infection lead to atherosclerosis or does atherosclerosis lead to infection? Additional temporal issues related to the age of subjects at the time of measurement and the timing of the measurement in relation to the onset of clinically recognized events, such as MI, also may need to be considered when interpreting the findings from seroepidemiologic studies. If \textit{C. pneumoniae} is related to the early stages of atherosclerosis, initiation, and progression, it may be necessary to obtain serologic measures early in life. On the other hand, if reinfection is the exposure associated with an increased risk of atherothrombotic disease, then recent measurements of high-titer antibody might be associated with an increase in risk.

Potential uncontrolled bias is another factor that needs to be considered when interpreting prior seroepidemiologic studies. Most studies take into account the potential bias related to older age, male sex, low socioeconomic status, and cigarette smoking, factors that are commonly related both to the prevalences of antibody (seropositivity and titer) and the risk of atherothrombotic disease. However, it is possible that other measures that reflect the dose of smoking, such as the age of initiation of smoking, pack-years, and exposure to passive smoking, are needed to fully account for the possible confounding influences of smoking.

The lack of consistency of the findings from seroepidemiologic studies of infection and atherosclerotic risk also may relate to differences in the characteristics of the study sample. It is possible that other factors related to atherothrombotic disease, such as elevated serum blood cholesterol and cigarette smoking, influence the atherosclerotic risk associated with infection. It also is possible that other factors contribute to the risk of clinical disease among persons with atherosclerosis. Across populations, the associations of infection with the same stage of atherothrombotic disease may differ, because of differences in the prevalences of other factors. Within the same population, infection may be related to the presence of subclinical atherosclerosis but not the occurrence of clinical events.

Given current limitations of seroepidemiology and on-going secondary prevention trials of antibiotic therapies, some have questioned whether further seroepidemiologic studies are likely to contribute important information regarding this possible association. In our view, seroepidemiology should help guide the development of clinical trials and complement animal experimental, pathologic, and molecular studies. To contribute new information, seroepidemiologic studies must be conducted in potentially informative populations, such as the young, with specification of the outcome, such as the stage of atherothrombotic disease. Further examination of the possible associations of atherothrombotic disease with high-titer IgG and IgA antibodies are needed, since high titers may reflect exposure to persistent, chronic active, or reinfection rather than merely “prior infection.” In addition, when available, the associations of circulating \textit{C. pneumoniae} antigen in monocytes with various atherothrombotic outcomes should be examined in population-based epidemiologic studies. Epidemiologic studies also are needed to explore further whether other factors (e.g., serum cholesterol and exposures to cigarette smoke over the life span) influence the atherosclerotic risk associated with \textit{C. pneumoniae}. Finally, it will be important for epidemiologic studies to consider the potential interrelationship of infection, inflammation, and atherothrombotic disease.

Taken together, research using a variety of research paradigms suggests a possible etiologic relation of infection with \textit{C. pneumoniae}.
pneumoniae and atherosclerotic risk. While the findings of seroepidemiologic studies appear inconsistent, alternative explanations may explain the apparent differences. It is possible that differences in the populations studied, the measures of prior exposure to infection with C. pneumoniae, the age at which antibody was assessed, and the atherothrombotic outcomes examined may account for the differences. Further research should explore potential alternative explanations by examining more fully the associations of high-titer antibody and, potentially, the presence of circulating C. pneumoniae antigens with different stages of the atherothrombotic process in the presence and absence of other factors and at different ages. Whether C. pneumoniae is associated with the early lesions of atherosclerosis that are common throughout the world, the progression of atherosclerotic lesions, or triggers plaque rupture later in life remains unknown. Thus, the design of seroepidemiologic studies should be guided by the pathophysiology of atherothrombotic disease and the findings from other research paradigms. Then the findings of seroepidemiologic studies and other research paradigms should be used to guide the design of clinical trials.

References