Lack of Association of Infectious Agents With Risk of Future Myocardial Infarction and Stroke
Definitive Evidence Disproving the Infection/Coronary Artery Disease Hypothesis?

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Over the past decade, increasing evidence has accumulated implicating a role for infectious agents in the genesis of atherosclerosis. Although far short of definitive, the increasing number of articles suggesting a causal role has led to the perception the case is proven, even stimulating some physicians and the media to wonder whether antibiotics should now be given to patients with coronary artery disease (CAD). However, it must be appreciated that publication bias has the tendency to lead to far more “positive” studies being published (the positive results often deriving from poor study designs) than negative studies (often deriving from excellent study designs). One of the best examples of this publication bias in the area of infection and CAD is seen in the ROXIS (ROXithromycin Ischemic Syndromes) trial, which was preliminarily published in the Lancet as a “positive pilot randomized trial” and has recently been published in final form in the European Heart Journal as a negative study.1,2 Given this publication bias and the as-yet-unproven nature of the infection/CAD hypothesis, it would seem important to emphasize those studies containing results that appear to be at variance with the concept that infection can contribute to CAD.

Two such studies, important because they involve a large cohort and because they are prospective in design, have been published recently in Circulation.3,4 However, just as there is the danger of prematurely accepting the infection/CAD hypothesis as proven, there is also the danger of using the results of such apparently conclusive negative studies to peremptorily dismiss the potential role of infection. It is therefore the purpose of this analysis to help put these 2 recent studies into perspective: to develop the case that such studies, despite their excellent design, must still be evaluated critically because they may contain inherent limitations that can lead either to erroneous conclusions or to conclusions that, although true, are limited in terms of how broadly they can be applied to larger populations.

In these carefully designed and thoughtfully reasoned studies, Ridker and associates reported that in an apparently healthy middle-aged group of white physicians, prior infection with cytomegalovirus (CMV), herpes simplex virus, or Chlamydia pneumoniae does not predispose to future risk of myocardial infarction and stroke.1,4 Evidence of prior infection was determined by the presence of IgG antibodies directed against the specific pathogen. Banked blood obtained several years before end-point assessment was used as the prospective data reservoir to determine “admission” antibody status.

Many previous seroepidemiological investigations5,6 demonstrating an association between an infectious agent and CAD derived from cross-sectional studies in which evidence of exposure to the pathogen was ascertained after rather than before the development of atherosclerosis. Conclusions deriving from these cross-sectional studies can only be considered as hypothesis generating rather than hypothesis proving. The reason for this is that the study design does not permit establishment of any temporal relation between infection and disease onset, and it is difficult to account for unintentional selection biases and unknown confounding influences. For example, patients who already have an end point on entry into study (such as severe debilitating atherosclerotic disease) may actually be susceptible to infection, and therefore any assumption that infection causes atherosclerosis would be erroneous. Similar problems derive from retrospective studies, in which unintentional selection bias is often an important confounding influence. In contrast, the 2 studies by Ridker and associates were prospective in design, with nested cases and controls. The conclusions deriving from such a study design can be taken as much stronger evidence for proving or disproving the hypothesis being examined.

However, there are certain limitations of studies derived from the database used by the Ridker articles, the Physicians’ Health Study. One of the strengths and, at the same time, weaknesses of the data is that its study population is highly homogeneous; white male physicians. It is often pointed out that the benefit of studying a homogeneous population is that many confounding influences, such as different socioeconomic backgrounds, are eliminated, and the results are thereby less likely affected by unknown confounding influences. Although this is undoubtedly true, the flip side of this argument is that whatever results relate to the population being studied may not be representative of other populations. For example, other populations may have different immune responses to pathogen infection, based perhaps in part on

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genetic differences of nonwhite or nonmale populations or differences in socioeconomic status that could introduce important environmental factors that might alter pathogen-host interactions.

Evidence is beginning to emerge that host-pathogen interactions are important factors in determining whether or not a pathogen can influence the atherogenic process. These clinical end points are epidemiological studies is that cardiovascular events are used to define the presence of CAD. These facts must be considered before a conclusion derived from a homogeneous cohort is generalized to the broader population.

Another very important difference between Ridker and colleagues’ prospective studies and most of the published cross-sectional studies is that the end points that were evaluated differ fundamentally. The association that was assessed by most cross-sectional studies was that between infection and the presence of CAD, that is, the atherogenic process itself. The association assessed by Ridker and colleagues’ prospective studies was that between myocardial infarction and stroke, end points precipitated by thrombotic complications of atherosclerosis. Thus, if infection is causally related to the initiation or progression of the atherogenic process but not causally related to its thrombotic complications, such a key association would go unrecognized. Ridker and colleagues clearly recognized this potential limitation of their study and were careful to point it out in the discussion.

Furthermore, one of the problems of many prospective epidemiological studies is that cardiovascular events are used to define the presence of CAD. These clinical end points are used because of the difficulty in justifying performing coro-nary angiography in a totally asymptomatic group of individuals. However, many more individuals will actually have CAD than are indicated by the percentage of the cohort defined as abnormal only after clinical end points are manifest, an example of misclassification bias. This can lead to inaccurate conclusions, because seropositive individuals may have a relatively low incidence of events but a high prevalence of CAD, which would go undetected and therefore not be factored into the analysis.

Evidence compatible with this consideration can be found in a population-based cohort study in which the end point assessed was the presence of carotid artery thickening determined by carotid ultrasound examination.8 Carotid thickening was taken as a marker of subclinical atherosclerosis. In that study, blood stored 13 to 18 years before ultrasound assessment was analyzed for CMV seropositivity. The investigators found a graded relation between the odds of carotid thickening and the levels of CMV antibodies. Thus, this population-based cohort study, like the cross-sectional studies cited above, demonstrated a positive association between prior infection with CMV and atherosclerosis.

In addition, the recent studies of Muhlestein and associates9 suggest that 1 or more of the above factors present in the Ridker study did actually mask a significant association between CMV seropositivity and cardiovascular events. These investigators stored blood obtained from a group of individuals undergoing catheterization in whom the diagnosis of CAD was established angiographically. In the patients with documented CAD, CMV seropositivity was associated with an increase in the number of events documented during follow-up. It also correlated with elevated CRP levels. Both the Ridker and Muhlestein studies were prospective, and the end points measured were similar. The major differences between the studies were that all of the patients in the Muhlestein study had documented CAD, whereas the diagnosis of the control subjects in the Ridker article could not be known precisely. In addition, the Muhlestein population was much more heterogeneous than the highly homogeneous population analyzed in the Ridker studies. Which of these differences, or what other unknown differences, may account for the disparate results is uncertain at this time.

Atherosclerosis is a multifactorial disease that is influenced not only by the presence or absence of various risk factors but by genetic and environmental heterogeneity. As a result, it is very difficult to establish a causal role for any single potential factor. This is dramatically illustrated by how long it took, and how many studies were involved, until it was finally generally accepted that elevated cholesterol levels and smoking are etiologically important factors in atherogenesis. These proofs depended on multiple lines of evidence. In terms of the evidence that is accumulating to link infection to atherogenesis, there have been many in vitro studies demonstrating that various infectious agents can alter the biological processes of cells of the vessel wall in ways that would appear to predispose to atherosclerotic lesion development.10–13 Moreover, in ApoE knockout mice (a genetic manipulation leading to the spontaneous development of atherosclerosis),14 it recently has been demonstrated that infection with C pneumoniae increases the progression of atherosclerosis. Such
proof of causality in this animal model provides compelling evidence that infectious agents have the capacity to contribute to atherosclerosis.

However, the studies by Ridker and associates do raise cautionary flags relating to the premature drawing of conclusions about any causal role of infection in the development of human atherosclerosis. Clearly, more such carefully controlled prospective studies on diverse patient populations are needed. What we have attempted to point out is that such studies can only be taken as one piece of the emerging and increasingly large totality of evidence. In the end, the hope of achieving definitive conclusions about the intriguing infection-atherosclerosis hypothesis is probably an elusive goal given the complexity of the disease, the multitude of pathogens that may contribute to the disease, and the complexity of host-pathogen interactions. Perhaps a more realistic goal we might hope to eventually achieve is to agree simply that there exists a high probability of causality. However, even this modest conclusion can only be accepted if additional studies on pathogen-induced disease-related mechanisms, multiple prospective seroepidemiological studies of different populations, additional investigations using animal models of disease, and human studies demonstrating that pathogen-targeted therapy reduces disease incidence or manifestations, convey reasonably consistent evidence linking infection to atherogenesis.

References


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