Chlamydia pneumoniae in Ischemic Heart Disease

Eduard Kaykov MD[1], Benyamine Abbou MD[1], Scott Friedstrom MD[2], Doron Hermoni MD[1] and Nathan Roguin MD[3]

Departments of [1]Family Medicine, [2]Infectious Diseases and [3]Cardiology, Western Galilee Hospital, Nahariya, and Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

Key words: *Chlamydia pneumoniae*, coronary artery disease, antibodies

See also page 272

Abstract

Background: Previous work has suggested an association between *Chlamydia pneumoniae* infection and coronary artery disease. The infection was demonstrated by titers of antibodies — enzyme-linked immunosorbent assay or immunofluorescence, and polymerase chain reaction — and by the findings of *C. pneumoniae* in the atherosclerotic plaque.

Objectives: To evaluate the association between chronic infection with *C. pneumoniae*, as measured by a high titer of IgG antibody, and CAD. Our study was designed to explore the relationship between seropositivity to *C. pneumoniae* and serious coronary events, and to assess whether or not there may be an additional association between established cardiovascular factors and infection with this organism.

Methods: The serum of 130 patients with proven CAD was tested for the presence of IgG antibodies to *C. pneumoniae* using an ELISA test. A titer ≤1:64 using the microinfluorescence method, the recognized "gold standard," correlates with a positive result when using the ELISA method. The mean age was 57 (40–65 years). The patients, 82% male and 18% female, had either myocardial infarction (n=109) or unstable angina (n=21) 6 months before the investigation (range 3–24 months). The serum for the control group was obtained from 98 blood donors from the same area matched for age 52 (40–58 years) and sex. The donors had no known cardiac history.

Results: In the CAD group 75% of patients were positive for *C. pneumoniae* compared to 33% in the control group ($P=0.001$). No increased correlation could be demonstrated between traditional risk factors and *C. pneumoniae* infection, except in those patients with diabetes mellitus. We found a lower prevalence of IgG antibody to *C. pneumoniae* in the diabetes subgroup than in other subgroups ($P<0.006$), but a higher prevalence than in the control group.

Conclusions: We demonstrated a more than twofold increase in seropositivity to *C. pneumoniae* among patients suffering serious coronary events, and this trend was independent of gender, age or ethnic group. These findings suggest that chronic *C. pneumoniae* infection may be a significant risk factor for the development of CAD, but this correlation should be investigated further.

IMAJ 1999;1:225–227

Established cardiovascular risk factors — such as cigarette smoking, diabetes mellitus, hypertension, hypercholesterolemia, age, gender and family history — do not fully explain the temporal and geographic variations in the prevalence of coronary artery disease. The potential role of infection in the development of atherosclerosis, the pathologic basis for CAD, was suggested by Sir William Osler and others at the beginning of this century [1]. Recent evidence indicates the possible role of viral and bacterial infections in the development of atherosclerosis and their association with myocardial infarction [2–7]. *Chlamydia pneumoniae*, a common intracellular pathogen frequently involved in upper and lower respiratory tract infections that are usually subclinical and self-limiting, has been recently associated with CAD [8,9].

Since *C. pneumoniae* is difficult to culture, confirmation of infection often requires identifying systemic antibody responses. About half of the population is seropositive to *C. pneumoniae* by age 50, suggesting that reinfection is common [10,11]. In Finland, investigators [8] discovered that in nearly 70% of paired sera taken from patients with acute myocardial infarction there was a seroconversion against the C.LPS antigen 4 weeks after the event. The patients with AMI had elevated immunoglobulin G and IgA

CAD = coronary artery disease

AMI = acute myocardial infarction

antibody titers against the pathogen. Elevated antibody levels to *C. pneumoniae* were also demonstrated in AMI [12,13] and in CAD [14]. In addition, in the Helsinki Heart Study the authors demonstrated the presence in sera of chlamydial LPS immunocomplexes as a risk factor for CAD [15].

The aim of the present study was to assess the role of *C. pneumoniae* infection in CAD in our population and to seek possible relationships between patients with this infection and established CAD risk factors.

Methods

Study population

We performed a retrospective cross-sectional study in 131 patients 3 to 24 months after myocardial infarction (n=117) or unstable angina (n=14) [Table 1]. Patients were recruited from the cardiology department of the Western Galilee Hospital from May 1998 to October 1998; 108 (82%) were male and 23 (18%) were women, and the mean age was 57 years (range 40–65). Ninety-two patients (70%) were Jews and 39 (30%) were Muslim, Christian or Druze. The sera of 98 blood donors from the same geographic area, matched for sex, age and ethnic group, were used as controls [Table 2]. No patients had history or symptoms of CAD. The blood donors' serum was taken in October 1998.

Diagnostic methods

Anti-*C. pneumoniae* antibodies of the IgG isotype were detected in both the patient and the control group with a new enzyme-linked immunosorbent assay: Sero CP (Savyon Diagnostics, Ashdod, Israel). The Sero CP plates are pre-coated with *C. pneumoniae* specific elementary bodies. The tested serum is diluted (1/105) and incubated with the Sero CP plates. The *C. pneumoniae*-specific antibodies bind to the plate. After washing the plate, anti-human IgG conjugated to horseradish peroxidase is added and binds to the antigen-antibody complex. After washing the plate again, HRP3 substrate is added. The substrate is hydrolyzed by the peroxidase, yielding a blue solution. The blue color turns yellow and can be read by an ELISA reader at 450 nm wave length. The cut-off value is calculated based on the results of internal kit controls matched to a titer of 1/64 as measured by microimmunoflurescence.

Statistical methods

Epidemiologic evaluation of our population was performed with SSPI-DOS 5.01, 1991. We used 2x2 tables and related chi-square with Levene's test for equality of variances. We used the Mantel-Haenszel test for linear association and Fisher's exact two-tail test. For independent samples we used the Wilcoxon rank sum W test.

Table 1. Coronary heart disease characteristics of the study group

Months after the cardiac event	6% (3–24)
Anterior MI	31% (37)
Inferior MI	46% (54)
Other MI	22% (26)
Unstable angina	10% (14)

Table 2. Demographic characteristics of the study and the control groups

Characteristics	Study group (n=131)	Control group (n=98)
Gender		
Male	82% (108)	78% (75)
Female	18% (23)	22% (23)
Mean age	57±7	52±8
Ethnic group		
Jews	70% (92)	78% (75)
Non-Jews	30% (39)	22% (23)

Table 3. The prevalence of *C. pneumoniae* IgG antibodies in the study and control groups

ELISA results	Study group (n=131)	Control group (n=98)	*P*
Positive	75.6% (99)	33% (32)	< 0.001
Negative	24% (32)	66.7% (64)	

Table 4. The correlation between high antibodies titer and major risk factors for CAD in the study group

Risk factor	Cases	*P* compared with CAD group
Diabetes mellitus	28% (37	0.006
Positive CP	59.5% (22)	
Negative CP	40.5% (15)	
Hypertension	59% (78)	NS
Positive CP	73.4% (58)	
Negative CP	26.6% (20)	
Dyslipidemia	51% (68)	NS
Positive CP	73.5% (50)	
Negative CP	26.5% (18)	
Smoking	55% (72)	NS
Positive CP	77.8% (56)	
Negative CP	22.2% (16)	
Family history	9% (12)	NS
Positive CP	83.3% (10)	
Negative CP	16.7% (2)	
BMI >27	21% (27)	NS
Positive CP	74.1% (20)	
Negative CP	25.9% (7)	

NS = not significant

Results

The prevalence of *C. pneumoniae* IgG antibodies in the study and the control group is summarized in Table 3. We found high levels of antibodies in the study group compared with the control group: 75.6% vs. 33% ($P<0.001$). There was no difference between the positive results according to gender or ethnic group. We did not observe a positive correlation between the presence of high antibody titer and major risk factors for CAD in the study group, except in those patients with diabetes mellitus. We found a low

HRP = horseradish peroxidase

prevalence of IgG antibody to *C. pneumoniae* in the diabetes mellitus subgroup compared to the other subgroups ($P<0.006$) [Table 4].

Discussion

Traditional cardiovascular risk factors, including smoking, diabetes, hypertension, family history and hypercholesterolemia, play an incontrovertible role in the development and progression of atherosclerosis, and modification of these risk factors can favorably alter the natural history of coronary artery and cerebrovascular disease. However, clinicians commonly care for patients suffering cardiovascular events in whom none of the established risk factors can be identified. Additionally, risk factor modification does not prevent disease. It is clear that our understanding of the pathogenesis of these diseases is incomplete.

Though the triggers remain unclear, there is ever-increasing clinical and experimental evidence that inflammation and immunological mechanisms play a major role in atherogenesis [16,17]. Evidence linking infection to cardiovascular disease has amassed since the 1970s, though this idea was first proposed by Sir William Osler at the beginning of this century. There are several candidate infectious agents that may serve as an inflammatory trigger, but recent work has focused on *C. pneumoniae*. Our study was designed to explore the relationship between seropositivity to *C. pneumoniae* and serious coronary events, and to assess whether or not there may be additional associations between established cardiovascular factors and infection with this organism.

We demonstrated a more than twofold increase in seropositivity to *C. pneumoniae* among patients suffering serious coronary events, and this trend was independent of gender, age or ethnic group. Israel's population is extremely diverse, comprising peoples from 150 ethnic groups. Several studies have demonstrated a similar increase in seroprevalence among cardiac patients but ours is the first study demonstrating this trend across such a broad ethnic population base.

We did not find a correlation between traditional risk factors and *C. pneumoniae*, except in those patients who had diabetes mellitus. There are proposed mechanisms of action by which infectious agents may contribute to atherogenesis. Infection with *C. pneumoniae* might induce a chronic immune activation, mediated by cytokines such as interleukin-1, tumor necrosis factor-alpha and IL-6 [18], that contributes to direct chronic endothelial cell damage or stimulates the synthesis of acute phase reactants such as fibrinogen [19] and C-reactive protein [20]. Chronic infection might also increase expression of monocyte-derived procoagulants such as tissue factor [20] and thereby increase the risk of local or distant thrombosis.

The idea that infection may play a role in coronary heart disease should not be viewed as opposing or in any way negating the classic coronary risk factors. Although to date the data linking CAD and infections do not prove causality, the ever-accumulating mass of data strongly implies a more than coincidental link. Our data reinforce the dramatic association between seropositivity to *C. pneumoniae* and serious coronary events. We will continue our investigation on other patients diagnosed with CAD and assess the effect of antibiotics on CAD.

References

1. Osler W. Disease of the arteries. In: Osler W, ed. Modern Medicine: Its Practice and Theory. Philadelphia: Lea & Febiger, 1908:429–47.
2. Pesonen E, Siitonen O. Acute myocardial infarction precipitated by infectious disease. *Am Heart J* 1981;101:512–13.
3. Spodick DH, Flessas AP, Johnson MM. Association of acute respiratory symptoms with onset of acute myocardial infarction: prospective investigation of 150 consecutive patients and matched control patients. *Am J Cardiol* 1984;53:481–2.
4. Mattila KJ. Viral and bacterial infections in patients with acute myocardial infarction. *J Intern Med* 1989;225:293–6.
5. Nieminen NS, Mattila K, Valtonen V. Infection and inflammation as risk factors for myocardial infarction. *Eur Heart J* 1993;14(Suppl k):12–16.
6. Woodhouse PR, Khaw KT, Plummer M, Foley A, Meade TW. Seasonal variations of plasma fibrinogen and factors VII activity in the elderly: winter infections and death from cardiovascular disease. *Lancet* 1994;343:435–9.
7. Dahlen GH, Boman J, Birgander LS. Lp(a) lipoprotein, IgG, IgA, and IgM antibodies to C. pneumoniae and HLA class II genotype in early coronary artery disease. *Atherosclerosis* 1995;114:165–74.
8. Saikku P, Leinonen M, Mattila K. Serologic evidence of an association of a novel chlamydia, TWAR, with chronic heart disease and acute myocardial infarction. *Lancet* 1988;29:983–6.
9. Saikku P, Leinonen M, Tenkanen L . Chronic C. pneumoniae infection as a risk factor for coronary heart disease in the Helsinki Heart Study. *Ann Intern Med* 1992;116:273–8.
10. Grayston JT, Kuo CC, Campbell LA, Beneditt P. C. pneumoniae strain TWAR and atherosclerosis. *Eur Heart J* 1993;14:57–61.
11. Grayston JT, Campbell LA, Kuo CC. A new respiratory tract pathogen: C. pneumoniae strain TWAR. *J Infect Dis* 1990;161:618–25.
12. Saikku P. C. pneumoniae infection as a risk factor in acute myocardial infarction. *Eur Heart J* 1993;14:62–5.
13. Leinonen M, Linnanmaki E, Mattila K. Circulating immune complexes containing chlamydial lipopolysaccharide in acute myocardial infarction. *Microb Pathog* 1990;9:67–73.
14. Thom DH, Grayston JT, Siscovick DS, Wang SP, Weiss KS, Daling JR. Association of prior infection with Chlamydia pneumoniae and angiographically demonstrated coronary heart disease. *JAMA* 1992;268:68–72.
15. Bihner V. Atherosclerosis and the immune system. *Arch Intern Med* 1998;158:1395–6.
16. Mattila KJ, Valtonen VV, Nieminen MS, Asikaninen S. Role of infection as a risk factor for atherosclerosis, myocardial infarction, and stroke. *Clin Infect Dis* 1998;26:719–34.
17. Grayston JT, Kuo CC, Campbell LA, Benditt EP. Chlamydia pneumoniae, strain TWAR and atherosclerosis. *Eur Heart J* 1993;14:66–71.
18. Patel P, Carrington D, Strachan DP, Leatham E, Goggin P, Northfield TC, Mendall MA. Fibrinogen: a link between chronic infection and coronary heart disease. *Lancet* 1994;343:1634–5.
19. Mendall MA, Patel P, Ballam L, Strachan D, Northfield TC. C reactive protein and its relation to cardiovascular risk factors: a population based cross sectional study. *Br Med J* 1996;312:1061–5.
20. Leatham EW, Bath PM, Tooze JA, Camm AJ. Increased monocyte tissue factor expression in coronary disease. *Br Heart J* 1995;73:10–13.

Correspondence: Dr. E. Kaykov, P.O. Box 4420, Maalot 21057, Israel. Tel: (972-4) 997 4982; email: edikaykov@yahoo.com