Incidence of Immunoglobulin G Antibodies to *Chlamydia pneumoniae* in Acute Myocardial Infarction Patients*

Haim Ashkenazi MD[1,2], Bernard Rudensky PhD[3], Esther Paz MA[3], David Raveh MD[1], Jonathan A. Balkin MBBCh[2], Dan Tzivoni MD[2] and Amos M. Yinnon MD[1]

[1]Infectious Diseases Unit, [2]Department of Cardiology, and [3]Clinical Microbiology and Immunology Laboratory, Shaare Zedek Medical Center, Jerusalem (affiliated with Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva), Israel

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Abstract

Background: Recent studies have suggested a possible association between *Chlamydia pneumoniae* infection and coronary heart disease.

Objectives: To determine titers of antibodies to *C. pneumoniae* in patients with acute myocardial infarction compared with titers in several control groups.

Methods: This prospective case-control study investigated 209 individuals. We assessed the serum IgG antibody titers to *C. pneumoniae* in 57 consecutive patients admitted with AMI to our intensive coronary care unit during a 4 month period. A serum sample was drawn upon admission and after 6 weeks. Results were compared with those of four control groups: a) patients admitted with community-acquired pneumonia (n = 18), b) patients with community-acquired urinary tract infection (n = 42), c) patients with angiographically normal coronary artery disease (n = 44), and d) patients with stable coronary artery disease (n = 48). Serum immunoglobulin G antibody titers to *C. pneumoniae* were determined using standard micro-immunofluorescence technology.

Results: Of 57 patients with AMI, 32 (56%) had a high IgG titer to *C. pneumoniae* ($\geq$1:256) on the initial test, which remained unchanged (62%) after 6 weeks. The percentage of patients with high titers was significantly lower in the control groups: 5 of 18 patients (28%) in the pneumonia group ($P < 0.01$), 11 of 42 (26%) in the urinary tract infection group ($P < 0.01$), 11 of 44 (25%) with normal coronary arteries ($P < 0.01$), and 17 of 48 (35%) with stable chronic ischemic heart disease ($P < 0.05$).

Conclusion: The detection of high titers of IgG antibodies to *C. pneumonia* in many patients with AMI, compared to control groups, suggests that chronic *Chlamydia pneumoniae* infection plays a role in the pathogenesis of atherosclerosis and acute ischemic events.

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Recently, *Chlamydia pneumoniae* was recognized as a major cause of acute respiratory tract infections in humans [1–3]. In addition, there is growing evidence that this organism may be involved in the pathogenesis of atherosclerosis. Several studies involving different detection methods such as polymerase chain reaction, immunohistochemistry, electron microscopy, *in situ* hybridization and culture have identified the organism in atherosclerotic lesions in various arteries [4–8]. In addition, several, though not all seroepidemiological studies of patients with coronary heart disease have shown an association between antibodies to *C. pneumoniae* and atherosclerosis [9–12]. Questions were raised concerning the sensitivity and specificity of serologic tests and cross-reactivity among various chlamydial species.

We conducted a prospective seroepidemiologic study on all consecutive patients admitted with acute myocardial infarction during a 4 month period, and compared the titers of IgG antibodies to *C. pneumonia* with those of four control groups, with a ratio of almost three control patients for each study patient. We employed serologic technology that distinguishes reliably between the various chlamydial species.

Subjects and Methods

This seroepidemiological study was conducted at the Shaare Zedek Medical Center, a 550-bed university-affiliated general hospital, during a 4 month period (1 January 1999 to 30 April 1999). The study was prospective, controlled and blinded. The study cohort consisted of all consecutive patients with AMI admitted to the hospital's six-bed coronary care unit during the specified period. In order to assess the significance of *C. pneumoniae* antibody titers in the study patients, results were compared with those of four control groups. The first included patients with community-acquired pneumonia, without evidence or history of coronary artery disease; and the second group included patients with acute urinary tract infection, without evidence or history of coronary artery disease. These two control groups were chosen to assess the possibility of anamnestic recall of *C. pneumoniae* antibody during the acute inflammatory process associated with myocardial infarction. The patients were identified and enrolled in the emergency room. The third group comprised patients with angiographically normal coronary arteries. These individuals were chosen from

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AMI = acute myocardial infarction

the cardiology department's register. Although they had undergone coronary angiography for various reasons, they were the closest to healthy control patients with anatomically normal coronary arteries. The fourth group consisted of patients with stable ischemic heart disease, defined as having had a past AMI and having been in cardiac rehabilitation for at least 6 months (many for more than one year) with no present signs of angina pectoris. These patients were chosen from the hospital's cardiac rehabilitation program.

Initial blood sampling was performed in the emergency room on the day of admission. Convalescent samples were obtained 6 weeks later in two groups of patients: the study cohort of patients who had sustained AMI and the patients who had been admitted because of community-acquired pneumonia. The latter samples were drawn either at the patient's home or in the hospital's outpatient clinic. The samples were frozen at –20°C. The laboratory determination of *C. pneumoniae* titers was performed 6 weeks after the last patients with AMI had been enrolled, shortly after the last convalescent sera were obtained. Therefore, the longest any of the samples had been frozen was 6 months. All blood samples were tested at the same time. The test tubes were identifiable by code to ensure blinding of the laboratory technicians.

We chose to determine the IgG antibody titer because this is considered the optimal marker of chronicity and/or reactivation of *C. pneumoniae*. Antibody titers were determined with the micro-immunofluorescence technique, widely accepted because of its high quality performance and ability to distinguish among various chlamydial strains. Throughout the study the commercial kit manufactured by MRL Diagnostics (Cypress, CA, USA) was used, which has a sensitivity and a specificity of at least 90% each. Serologic tests, when positive, were titrated until a titer of at least 1:256. Fluorescent microscopy (Axioskop model, Zeiss, Switzerland) was carried out at a magnification of x400. The difference in *C. pneumoniae* IgG antibody titers was compared between the study cohort with AMI and each of the four control groups.

This study was approved by the hospital's Helsinki Committee and all patients gave informed consent.

Data were entered into the Excel spreadsheet. Analysis was conducted with the EPI-Info 6.0C program (CDC, Atlanta, GA, USA). For statistical evaluation of differences between the small groups we employed chi-square analysis, Student's *t* and Fisher's exact tests. Significance levels were set at $P < 0.05$.

Results

During the 4 month study period, 57 patients with acute myocardial infaction were admitted to the coronary intensive care unit. All patients were enrolled. The baseline demographic characteristics of the patients with AMI and the 152 patients of the four control groups are presented in Table 1. The IgG antibody titers to *C. pneumoniae* are presented in Table 2. The majority of patients in all groups (81 ± 12%, mean ± SD) had serological evidence of exposure to *C. pneumoniae*, with titers ⩾ 1:16. However, titers of IgG antibody *in excess* of 1:256 were detected in 56% of patients with AMI, as compared to 28% in patients with pneumonia ($P < 0.05$), 26% in patients with urinary tract infection ($P < 0.01$), 25% in patients with normal coronary arteries ($P < 0.01$), and 35% in patients with stable chronic ischemic heart disease ($P < 0.05$). The breakdown of serologic results by gender is shown in Table 3. IgG titers in excess of 1:256 were present in 5 of 14 female patients (36%) with AMI, compared to 18 of 72 (22%) female patients in the control groups (not significant). IgG titers in excess of 1:256 were present in 27 of 43 male patients (63%) with AMI, compared to 28 of 80 male patients in the control groups (35%) ($P < 0.001$). In the AMI group, 61% of patients with anti-chlamydia titers in excess of 1:256 were smokers compared to only 27% of patients with lower titers ($P = 0.02$). All of the smokers (n=19) were male.

The distribution of IgG seropositivity among the various standard dilutions (i.e., 1:16, 1:28, 1:64 and 1:256) was reexamined in the AMI study cohort and in the pneumonia control group by means of repeat sampling 6 weeks after the initial testing. No seroconversion, a hallmark of acute infection, emerged. The percentage of AMI patients with an IgG titer of 1:256 increased non-significantly from 56% at baseline to 62% at repeat testing. In patients with pneumonia, 5 of 18 patients (27%) had an IgG titer of 1:256 at baseline compared to 4 of 18 (22%) at follow-up. Thus, reexamination of these two groups of patients at 6 weeks revealed serologic stability.

Table 1. Breakdown by age, gender and diagnosis of 209 participating patients (n,%)

Group	n	Age ⩽ 55 yr		Age > 55 yr	
		Male	Female	Male	Female
Acute MI	57	14 (25)	0	29 (51)	14 (24)
Pneumonia	18	1 (5)	0	10 (56)	7 (39)
UTI	42	1 (2)	9 (21)	11 (26)	21 (50)
Normal CA	44	11 (25)	12 (27)	7 (16)	14 (32)
CIHD	48	9 (19)	1 (2)	30 (62)	8 (17)
Total	209 (100)	36 (17)	22 (10)	87 (42)	64 (31)

UTI = urinary tract infection, CA = coronary angiogram, CIHD = chronic ischemic heart disease.

Table 2. IgG antibody titers to *C. pneumoniae* in various groups (n, %)

Group	n	Seronegative	Seropositive: IgG titers			
			⩾16	⩾1:64	⩾1:128	⩾1:256*
Acute MI	57	6 (10)	51 (89)	43 (75)	34 (60)	32 (56)
Pneumonia	18	1 (6)	17 (94)	15 (83)	10 (56)	5 (28)
UTI	42	13 (31)	29 (69)	25 (60)	19 (45)	11 (26)
Normal CA	44	15 (34)	29 (66)	20 (45)	13 (29)	11 (25)
CIHD	48	7 (15)	41 (85)	31 (65)	18 (38)	17 (35)
Total	209	42 (20)	167 (80)	134 (64)	94 (45)	76 (36)

* The difference in seroprevalence of high IgG titers (⩾1:256) between the acute MI group and the following groups was significant: pneumonia ($P < 0.05$), UTI ($P < 0.01$), normal coronary artery ($P < 0.01$), and chronic ischemic heart disease ($P < 0.05$).

Table 3. Patients with IgG titers of 1:256: Breakdown by gender (n, %)

Group	n (%)	Female	Male	Total*
Acute MI	57	5/14 (36)	27/43 (63)	32/57 (56)
Pneumonia	18	2/7 (28)	3/11 (27)	5/18 (27)
UTI	42	6/30 (20)	5/12 (42)	11/42 (26)
Normal CA	44	6/26 (23)	5/18 (28)	11/44 (25)
CIHD	48	2/9 (22)	15/39 (38)	17/48 (35)
Total	209 (100)	21/86 (24)	55/123 (45)	76/209 (36)

* *P* value: the difference between patients with acute MI and the other diagnostic groups was significant ($P < 0.05$–$P < 0.01$, see Table 2).

Discussion

In this prospective seroepidemiological study we compared all 57 patients consecutively admitted with acute myocardial infarction during a 4 month period to 152 control subjects (four groups). Of these patients 58% had very high IgG antibody titers (>1:256) to *Chlamydiae pneumoniae*. This percentage was significantly higher than that in any of the four control groups (25–35%, $P < 0.05$-0.01) and would seem to suggest a role for *C. pneumoniae* in the etiology. Although our study sample was small, this weakness was offset by the inclusion of four control groups. Control groups included patients with pneumonia (n=18), urinary tract infections (n=42), chronic stable ischemic heart disease (n=48), and individuals with angiographically normal coronary arteries (n=44). We included control groups with pneumonia and urinary tract infection to rule out the possibility that the inflammatory response itself, as seen in these conditions, as well as in acute MI, could raise IgG levels simply due to polyclonal B cell activation. Our findings appeared to refute this possibility.

A possibility also exists that the titers might be even higher in AMI patients but that some of the *chlamydia*-specific antibody adsorbed onto chlamydial antigen in the infarcted area. This phenomenon has been described by Hoppichler et al. [13] regarding antibodies to heat shock proteins.

These findings are not in accord with an earlier and larger (n=302) Jerusalem-based study, published by Kark et al. [12], which did not detect a distinguishing chlamydial antibody response among patients with known cardiovascular disease. However, several factors may explain the different results. First, our study population consisted of patients with *acute* myocardial infarction, whereas the previous study included patients with known cardiovascular disease. In our study, 35% of the control group with chronic stable ischemic heart disease had IgG titers $\geqslant$ 1:256, which was marginally higher than in the other three control groups, but still significantly lower than in the patients with AMI. Second, the serum samples in our study were examined relatively soon after they had been obtained, whereas in Kark's study they could have been frozen for longer periods. Third, in our study serum antibody titers were end-titrated and the results of the AMI patients were compared with those of four control groups, including patients with pneumonia. The vast majority of patients in all groups (81% $\pm$ 12, mean SD) had serological evidence of exposure to *C. pneumoniae*, with titers $\geqslant$ 1:16, which concurs with data published by Lieberman and coworkers [14] on the high prevalence of pneumonia due to this organism in Israel.

The results of our study are similar to those reported by Kaykov et al. from Israel [11]. In the latter study, 75% of 131 AMI patients had high antibody titers to *Chlamydia*, compared to only 33% of 98 control patients. However, there are important differences in design between these two studies. Kaykov's study was retrospective, used one sample per patient that was drawn weeks to months after the coronary event, compared with one control group of uncertain quality, and relied on a new enzyme-linked immunosorbent assay test. In our prospective study we carefully chose the control groups. Two samples were obtained: on admission with AMI and after a 6 week interval. In spite of these methodologic differences, the results are similar and indicate a high seroprevalence of *Chlamydia* infection in Israeli patients with acute or recent coronary events as compared to control patients.

In addition to the conflicting results from various sero-epidemiological surveys, it remains to be proven whether the association between *C. pneumoniae* and coronary artery disease is etiological. The identification of the organism in vascular tissue by electron microscopy, polymerase chain reaction, immunocytochemical staining, and culture provides further evidence for a causal relationship. Infection of rabbits has been shown to accelerate the development of atheromatous lesions in the aorta. However, several studies have failed to show a correlation between *C. pneumoniae* infection and atherosclerosis. A German team [15] recently reported its failure to find either an important bacterial presence in 50 atherectomy specimens or a significant antibody response to *Chlamydia* in the patients under study. In a much larger (n=1,034) study of antibody prevalence in patients with carotid atherosclerosis, seropositivity to the bacterium (IgG in 58% and IgA in 32%) was associated with already known risk factors for cardiovascular disease, but no independent association with heart disease emerged [16]. A recent study, although showing a possible association between atherothrombosis and herpes simplex virus type 1 infection, failed to show this association with chlamydial infection except in patients with extremely high titers (> 1:1,024) [17].

Anti-*Chlamydia* antibiotic trials (usually with macrolides) have been undertaken that used a decrease in event rates (second MI, cardiac death, anginal attacks) as endpoints. Encouraging early results have been reported by Gupta et al. [18] from London, who documented a decrease in event rates (with and without lowered chlamydial antibody titers) after administration of azithromycin to 40 post-MI patients. Similar results were reported by Gurfinkel et al. [19], showing a significant reduction in serious cardiac events and associated mortality in patients treated with roxithromycin. Several other larger antibiotic trials are now underway [20].

Certain results in our study deserve further comment. Firstly, the AMI group had significantly higher antibody titers than the patients with stable coronary artery disease, and these titers did not appreciably change during the 6 weeks post-acute event.

The possibility exists that during the acute event chlamydial antigen might be released during rupture of the atherosclerotic plaque(s), resulting in a booster immunologic response with increased antibody titer. As we only titered to 1:256 during the acute and convalescent stages, we were unable to determine dynamics above this titer level. This certainly should be looked at in any future study.

Secondly, the serological association of AMI with *Chlamydia* infection was very strikingly related to smoking. A significantly higher percentage of AMI patients with high titers were smokers as compared to patients with lower titers. A possible explanation could be that smoking might be a risk factor for acute chlamydial respiratory disease, which in some patients subsequently develops into chronic infection of the cardiovascular system.

Thirdly, our results suggesting a role for *C. pneumoniae* in AMI appear to be relevant to men rather than to women, in whom we did not see the same significant serologic response. It is possible that *C. pneumoniae* infection is just as relevant in women as in men but that our sample size was too small to detect this. Another possibility is that *C. pneumoniae* is a risk factor only in men, and that in women other factors are much more significant. A third possibility is that the significance in males is simply a reflection of the higher prevalence of male smokers. This possibility gains credence in light of our findings regarding smoking. In our study, all the smokers were male, and if smoking is truly a contributing factor to *Chlamydia*-associated AMI the male associated risk factor can be better understood.

In conclusion, this seroepidemiologic survey provides further evidence of the association of *C. pneumoniae* infection with atherosclerotic cardiovascular disease, especially in male patients who are smokers. Atherosclerosis is an inflammatory condition in which multiple factors have been shown to be involved; chronic infection could be an initiator, catalyst, or innocent bystander in this process. The epidemic proportions of cardiovascular diseases in the modern world mandate further study of the possible causal relationship between *C. pneumoniae* and cardiovascular diseases [21,22].

References

1. Grayston JT, Kuo CC, Wang SP, Altman J. A new *Chlamydia psittaci* strain, TWAR, isolated in acute respiratory tract infections. *N Engl J Med* 1986;315:161–8.
2. Grayston JT. Infections caused by *Chlamydiae pneumoniae* strain TWAR. *Clin Infect Dis* 1992;15:757–61.
3. Kuo CC, Jackson LA, Campbell LA, Grayston JT. *Chlamydiae pneumoniae* (TWAR). *Clin Microbiol Rev* 1995;8:451–61.
4. Blasi F, Denti F, Erba M, Cosentini R, Raccanelli R, Rinaldi A, Fogetti L, Esposito G, Rubetti L, Allegra L. Detection of *Chlamydiae pneumoniae* but not *Helicobacter pylori* in atherosclerotic plaques of aortic aneurysms. *J Clin Microbiol* 1996;34:2766–9.
5. Kuo CC, Shor A, Campbell LA, Fukushi H, Patton DL, Grayston JT. Demonstration of *Chlamydiae pneumoniae* in atherosclerotic lesions of coronary arteries. *J Infect Dis* 1993;167:841–9.
6. Kuo CC, Grayston JT, Campbell LA, Goo YA, Wissler RW, Benditt EP. *Chlamydiae pneumoniae* (TWAR) in coronary arteries of young adults (15-34 years old). *Proc Natl Acad Sci USA* 1995;92:6911–14.
7. Grayston JT, Kuo CC, Coulson AS, Campbell LA, Lawrence RD, Lee MJ, Strandness ED, Wang SP. *Chlamydiae pneumoniae* (TWAR) in atherosclerosis of the carotid artery. *Circulation* 1995;92:3397–400.
8. Kuo CC, Gown AM, Benditt EP, Grayston JT. Detection of *Chlamydiae pneumoniae* in aortic lesions of atherosclerosis by immunocytochemical stain. *Arterioscler Thromb* 1993;13:1501–4.
9. Saikku P, Leinonen M, Matilla K, Ekman MR, Nieminen MS, Makela PH, Huttenen JK, Valtonen V. Serologic evidence of an association of a novel Chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. *Lancet* 1988;ii:983–6.
10. Saikku P, Leinonen M, Tenkanen L, Linnanmaki E, Ekman MR, Manninen V, Manttari M, Frick MH, Huttenen JK. Chronic *Chlamydiae pneumoniae* infection as a risk factor for coronary heart disease in the Helsinki Heart Study. *Ann Intern Med* 1992;116:273–8.
11. Kaykov E, Abbou B, Friedsform S, Hermoni D, Rogin N. *Chlamydia pneumoniae* in ischemic heart disease. *IMAJ* 1999;1:225–7.
12. Kark JD, Leinonen M, Paltiel O, Saikku P. *Chlamydia pneumoniae* and acute myocardial infarction in Jerusalem. *Int J Epidemiol* 1997; 26:730–8.
13. Hoppichler F, Lechleitner M, Traweger C, Schett G, Dzien A, Sturm W, Xu Q. Changes of serum antibodies to heat-shock protein 65 in coronary heart disease and acute myocardial infarction. *Atherosclerosis* 1996;126:333–8.
14. Lieberman D, Schlaeffer F, Boldur I, Lieberman D, Horowitz S, Friedman MS, Leinonen M, Horovitz O, Manor E, Porath A. Multiple pathogens in adult patients admitted with community-acquired pneumonia: a one year prospective study of 346 consecutive patients. *Thorax* 1996:52;179–84.
15. Jantos CA, Nesseler A, Waas W, Baumgartner W, Tillmanns H, Haberbosch W. Low prevalence of *Chlamydia pneumoniae* in atherectomy specimens from patients with coronary heart disease. *Clin Infect Dis* 1999;28:988–92.
16. Coles KA, Plant AJ, Riley TV, Smith DW, McQuilleam BM, Thompson PL. Lack of association between seropositivity to *Chlamydia pneumoniae* and carotid atherosclerosis. *Am J Cardiol* 1999;84:825–8.
17. Siscovick DS, Schwartz SM, Corey L, Grayston JT, Ashley R, Wang SP, Psaty BM, Tracy RP, Kuller LH, Kronmal RA. Chlamydia pneumoniae, herpes simplex virus type 1, and cytomegalovirus and incident myocardial infarction and coronary heart disease death in older adults: The cardiovascular health study. *Circulation* 2000;102:2335–40.
18. Gupta S, Leatham E, Carrington D, Mendall M, Kaski JC, Camm J. Elevated Chlamydia pneumoniae antibodies, cardiovascular events, and azithromycin in male survivors of myocardial infarction. *Circulation* 1997;96:404–7.
19. Gurfinkel E, Bozovich G, Daroca A, Beck E, Mautner B. Randomised trial of roxithromycin in non-q-wave coronary syndromes: ROXIS pilot study. *Lancet* 1997;350:404–7.
20. Smith D, Gupta S, Kaski JC. Chronic infections and coronary heart disease. *Int J Clin Pract* 1999;53:460–6.
21. Campbell LA. Chlamydia pneumoniae and cardiovascular disease. *Emerg Infect Dis* 1998;4:571–9.
22. High K. Atherosclerosis and infection due to Chlamydia pneumoniae or cytomegalovirus: weighing the evidence. *Clin Infect Dis* 1999;28:746–9.

Correspondence: Dr. A.M. Yinnon, Infectious Diseases Unit, Shaare Zedek Medical Center, P.O. Box 3235, Jerusalem 91031, Israel. Phone: (972-2) 655-5076, Fax: (972-2) 666-6840, email: Yinnon@SZMC.org.il