

RESEARCH ARTICLE**Acute *Chlamydomphila pneumoniae* infection and its association with cardiovascular diseases.****Authors**

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Conflict of interests

The authors declare that they have no conflict of interest.

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Abstract

Chronic infection with *Chlamydomphila pneumoniae* (*C pneumoniae*) has been associated with cardiovascular disease (CVD). This study evaluates the association of acute *C pneumoniae* infection and CVD in adult patients. We conducted a cross-sectional study that included 397 patients with a diagnosis of CVD, and 127 without CVD, at the *Hospital General de Zona del Instituto Mexicano del Seguro Social* (IMSS) in the state of Morelos, Mexico; the diagnosis was established by the Department of Cardiology. Patients with CVD were divided into 4 groups: I) Cardiac arrhythmia, II) Ischemic heart disease, III) Hypertensive heart disease and, IV) Valvular heart disease. Exposure to *C pneumoniae* was measured by antibody determination (IgG, IgM e IgA) with the microimmunofluorescence (MIF) technique. Statistical analysis included the determination of prevalence, and multiple logistic regression analysis. Results showed that the prevalence of acute *C pneumoniae* infection in the study population was 21%, and was highest in the groups with CVD *versus* patients without CVD, and an association was established (OR, 13.93; 95% CI, 3.8-66.1). Likewise, when comparing the Ig titer geometric means, we found that an acute *C pneumoniae* infection increased the risk of presenting a cardiac arrhythmia and ischemic heart disease by a factor of 18, and increased 12-fold the risk of having hypertensive or valvular heart disease. The prevalence of acute infection + chronic infection was greater in the group with ischemic heart disease (18.4%). Our study reflects the great significance of seroprevalence of acute infection with *C pneumoniae* among patients with CVD. Further, the seroprevalence of acute infection + chronic infection was greater in patients with ischemic heart disease, suggesting that this combination may be associated with the mechanisms leading to atherosclerosis mediating coronary episodes. It appears that this is the first report on the subject.

Keywords: Cardiovascular disease; *Chlamydomphila pneumoniae*; Immunoglobulins; Microimmunofluorescence.

1. Introduction

Cardiovascular disease (CVD) remains a major contributor to worldwide morbidity. In the USA, cardiovascular disease is the main cause of death, responsible for 1 of every 4 deaths.¹ Although it decreased considerably for three decades since the 1980's, the overall mortality rate due to CVD increased 3% between 2011 and 2014, even above cancer-related deaths.² To date, the total number of deaths due to CVD throughout the world, has also increased 45% from 12, 3 million (1990) to 17, 8 million (2019). Most (85%) deaths related to cardiovascular disease are accounted for by severe hypertension, myocardial infarction,

and stroke.^{3, 4} In Mexico, as of 2018, CVD was among the top ten causes of death. Ischemic heart disease led to 108,616 deaths, the first cause of mortality nationwide. Stroke was the fourth cause, accounting for 35,300, and finally, hypertensive heart disease was in eighth place, causing 23,715 deaths.⁵

CVD refers to a group of heart and blood vessel diseases that include: coronary artery disease, stroke, peripheral arterial disease, rheumatic heart disease, congenital heart disease, deep venous thrombosis, and pulmonary embolism,⁶ all important contributors to a decrease in quality of life.⁷

Local or systemic infections may compound the atherogenic process.⁸ The bacterial components of *Chamydophila pneumoniae* (*C. pneumoniae*) have been particularly associated with the genesis of atheromatous plaque, and the risk of developing myocardial infarction, since it has been found in atheromatous lesions using different methods including serology, immunocytochemistry, PCR, and *in situ* DNA hybridization.⁹⁻¹¹ The chronic inflammatory process is associated with the pathogenesis of coronary artery disease, endocarditis, myocarditis, pericarditis, atherosclerosis, and hypertension.^{12, 13}

C pneumoniae can spread through peripheral blood mononuclear cells, and replicate in vascular cells after entering the tissue; it may also promote the development and persistence of an inflammatory state through numerous intracellular signaling pathways, such as the nuclear factor κ B pathway. This compromises vascular endothelial cell function, mononuclear macrophages, smooth muscle cells, platelets, and induces oxidative stress thus injuring endothelial cells, and forming foam cells.¹⁴

On a national level, at the *Instituto Mexicano del Seguro Social* (IMSS) (Mexican Institute of Social Security) and the IMSS delegation in the state of Morelos, CVD is the main reason for medical consultation. In two previous studies in Mexico City, our group found a strong association in CVD patients with *C pneumoniae* (97%) IgG titers (chronic infection), only present in 37% of healthy individuals.¹⁵ Likewise, our group also documented the presence of IgG antibodies against *C pneumoniae* in 70% of patients with acute myocardial infarction.¹⁶ Therefore, this study's aim was to establish a possible association between acute *C pneumoniae* infection and CVD in Mexican adult patients.

2. Material and Methods

2.1 Study population

Between 2014 and 2016, we recruited a total of 397 adult patients with CVD, and 127 without CVD; data on heart disease patients was obtained in the Department of Cardiology of the *Hospital General de Zona Instituto Mexicano del Seguro Social* (IMSS) in the state of Morelos. Patients without CVD were recruited simultaneously in the Family Medicine Department of the same hospital. All patients completed a clinical and a demographic questionnaire; a blood sample and an electrocardiogram were also obtained. All ethical requirements for this investigation were fulfilled. All study subjects signed a consent form, and the study was approved by the Research Committees of the IMSS and the *Instituto Nacional de Salud Pública* (INSP) (National Institute of Public Health).

2.2 Inclusion Criteria.

We included patients aged 18 or above, entitled to the medical services of the IMSS at the *Hospital General de Zona del Estado de Morelos*, with a diagnosis of CVD. They had to provide a sufficient amount of peripheral blood to determine antibody titers against *C pneumoniae*, and answered the required questionnaire. Accepted comorbidities included: diabetes mellitus, systemic hypertension, dyslipidemia and rheumatic disease. The same criteria were applied to the population without CVD.

2.3 Data Collection

All participants completed a questionnaire that included clinical and demographic information, their family's medical history, and the most relevant personal pathological and non-pathological information; it also included information on their laboratory results. The questionnaire was applied at the time of recruitment. A surface, 12-lead electrocardiogram was obtained in all patients. Patients with CVD were divided

into groups that included: cardiac arrhythmia, ischemic heart disease, hypertensive heart disease, and valvular heart disease, on the basis of the diagnosis stated in their clinical chart, and established by the cardiologists at the IMSS following the Mexican diagnostic guidelines issued by the Ministry of Health. A blood sample was obtained from all participants, and forwarded to the Immunology and Infectious Disease Research Unit of the *Centro Médico Nacional (CMN) "La Raza"*, to blindly determine antibodies against *C. pneumoniae* by microimmunofluorescence (MIF).

2.4 Definition of exposure

Serologic testing was conducted blindly using the microimmunofluorescence (MIF) technique developed by Wang and Grayston, regarded as the serological gold standard.¹⁷ Elemental *C pneumoniae* bodies were used as antigens in the MIF assay, and IgG, IgM, and IgA were detected. *C trachomatis* and *C psittaci* antigens were included in the assay. Cross-reactions between the different chlamydia species and the immunological effect of LPS on the *C pneumoniae* and *C trachomatis* antigens appear to be low.

2.5 Principle of the Assay.

This assay is based on the indirect detection of IgG, IgM, and IgA antibodies against *C pneumoniae* using fluorescein isothiocyanate (FITC) as a marker. Antibodies against *C pneumoniae* in patient serum were combined with *C pneumoniae* antigens fixed on a slide. They were washed, FITC conjugated to anti-human antibodies were added, and results were read in an epifluorescence microscope. Sera were

doubly diluted, from 16 to 512 to determine IgG, 16 for IgM, and 16 to 64 for IgA.

An acute infection was established if the patient had an IgM titer of 16, or an IgG titer ≥ 512 ; a chronic infection was based on IgG titers ≥ 16 but ≤ 512 , and a persistent infection was characterized by an IgA titer ≥ 16 .^{18, 19}

3. Statistical Analysis: A descriptive analysis was applied to the results of the participating population's characteristics.

3.1 Bivariate Analysis: To determine differences between groups, we used non-parametric and parametric statistics, and presented the results as percentages, means, and standard deviations. To determine between-group clinical characteristics, we used the Chi² and corrected Student t for multiple comparisons.

3.2 Multiple Regression Analysis

Multiple logistic regression analysis was performed to determine the association of *C. pneumoniae* infection and CVD. The model was adjusted for the most relevant covariables.

4. Results

There was no difference in terms of gender between the groups with and without CVD. There was a difference, however, ($p < 0.001$), if a history of diabetes mellitus was present in the groups with CVD. Variables such as systemic hypertension and overweight-obesity yielded no differences between the groups with CVD. A history of rheumatic fever did establish a difference between patients with and without CVD ($p < 0.0001$) (Table 1).

Table 1. Population characteristics

Group of patients by diagnosis	Group without CVD ^a	Cardiac arrhythmia	Hypertensive heart disease	Ischemic heart disease	Valvular heart disease	p value
Number of patients	127 (24%)	83 (16%)	98 (19%)	156 (30%)	60 (11%)	
Men	69 (54.3%)	33 (39.8%)	41 (41.8%)	75 (48.1%)	26 (43.3)	p = 0.207 ^b
Women	58 (45.7%)	50 (60.2%)	57 (58.1%)	81 (51.9%)	34 (56.7)	p = 0.207 ^b
Age (mean, SD)	57.3 (15.5)	60.1(18.1)	63.8 (13.7)	64.1(13.1)	58.2 (16.5)	p = 0.014 ^c
Mellitus diabetes	2 (1.6%)	19 (22.9%)	27 (27.6%)	51 (32.7%)	11 (18.3%)	p = <0.001 ^b
Systemic hypertension	no data	59 (71.8%)	82 (83.7%)	120 (76.9%)	43 (71.7%)	p = 0.17 ^b
Rheumatic fever	no data	2 (2.4%)	6 (6.1%)	7 (4.5%)	18 (30%)	p<0.0001 ^b
Overweight-obesity	90 (73.8%)	69 (84.2%)	83 (86.5%)	126 (82.4%)	44 (74.5%)	p = 0.091

^a Cardiovascular disease^b chi²^c “t” test

The prevalence of acute *C. pneumoniae* infection was 21%, and was greater in the groups with CVD than in subjects without CVD (p =0.002); the group with ischemic heart disease had the greatest prevalence. In

terms of chronic infection, a difference was detected (p =0.017), whereby the group without CVD had the greatest prevalence followed by patients with ischemic heart disease (Table 2).

Table 2. Prevalence of infection by *Chlamydomphila pneumoniae*

Patients groups by Diagnosis	Group without CVD ^a	Cardiac arrhythmia	Hypertensive heart disease	Ischemic heart disease	Valvular heart disease	chi ²
Acute infection	2 (1.5%)	19 (22.8%)	16 (16.3%)	36 (23.1%)	10 (16.6%)	p = 0.002
Chronic infection	111 (87.4%)	60 (72.3%)	75 (76.5%)	125 (80.1%)	41(68.5%)	p = 0.017

^a Cardiovascular disease

Multiple logistic regression analysis adjusted for age, gender, and overweight-obesity to determine the association of *C pneumoniae* and CVD, showed the following result: the possibility of having

CVD increased 14-fold if the patient had an acute infection p= (0.0001). We must underscore the fact that the OR overestimates the prevalence ratios when the disease’s prevalence is greater than 10%.

With chronic infection, we observed a 53% decrease in the risk of having CVD ($p = 0.007$); this result may be secondary to a sample effect or to some population

characteristic (immunity) with a protective effect. When analyzed by group, patients without CVD had a greater prevalence of this type of infection. (Table 3).

Table 3. Association of *Chlamydomphila pneumoniae* infection and cardiovascular disease

	Acute infection		Chronic Infection	
Cardiovascular disease	OR	95% CI	OR	95% CI
	13.93	IC 95% (3.8 – 66.1)	0.47	IC 95% (0.25-0.800)
Valor p	0.0001		0.007	

As to the association of some form of CVD in the presence of an acute infection by *C. pneumoniae*, the adjusted multiple logistic regression analysis is shown in (Table 4). In the presence of a chronic infection, logistic regression analysis adjusted to age, gender, overweight-obesity, decreased by

58% the possibility of harboring a cardiac arrhythmia (OR, 0.42; 95% 95% CI, 0.18-0.76), and decreased by 68% the possibility of having valve disease (OR, 0.32; 95% IC 95%, 0.14-0.66); this result may be an effect of inverse causality.

Table 4. Estimation of the possibility of having some type of CVD^a vs not having CVD when acute infection by *Chlamydomphila pneumoniae* is present

	Cardiac arrhythmia	Hypertensive heart disease	Ischemic heart disease	Valvular heart disease
Acute infection				
OR	18.54	11.99	18.83	12.65
95% IC	4.1-82.15	2.7-54.4	4.4-79.5	2.6-59.0
p value ^b	0.0001	0.001	0.0001	0.001

^a Cardiovascular disease

^b Multiple logistic regression model adjusted for: age, gender, diabetes mellitus.

5. Discussion

The prevalence of acute infection with *C pneumoniae* in the study population was 21%, and was greater in the groups with CVD than without CVD, thus establishing an association (OR, 13.93; 95% CI, 3.8-

66.1). We found only one report in the literature referring an association of acute infection with coronary artery disease, but they used PCR instead of MIF to detect the 16 SrRNA gene of *C pneumoniae*.²⁰ Perhaps there is a paucity of reports because most

acute infections are asymptomatic or mildly symptomatic, and below the threshold warranting medical care.²¹ Most of the patients presented chronic infection by *C pneumoniae*, but the important finding was that in those who also presented acute infection, valvular heart disease and ischemic heart disease were associated by serology and with statistical significance. This combination can precipitate an acute coronary syndrome due to the instability of the atheroma plaque mediated by inflammatory processes.

5.1 Cardiac arrhythmia

There are various factors that may lead to the development of cardiac arrhythmias, and among them, are infectious factors causing myocarditis. On the basis of the implied role of inflammation associated with the resultant arrhythmias, a factor triggering inflammation has been sought. The hypothesis suggesting that bacterial infections play a role in the pathogenesis of atrial fibrillation (AF), has been recently posited; *C pneumoniae* is one of the bacteria that has raised interest since it has the same epidemic tendency as AF. Infection of endothelial cells with *C. pneumoniae* has been shown to trigger the production of monocyte chemoattractant protein-1, interleukin-1, interleukin-8, interleukin-18, tumor necrosis factor, interferon, and soluble intercellular adhesion molecule-1. Most cytokines play a crucial role in the inflammatory response associated with the initiation and maintenance of AF. There are many pathogens that can trigger inflammation but some evidence has shown that *C pneumoniae* is the most probable pathogen associated with AF.^{22,23}

When we conducted the multiple logistic regression analysis, we detected that acute infection with *C pneumoniae* increased the possibility of developing cardiac arrhythmias by a factor of 18, and there was no effect resulting from chronic infection.

Perhaps this is because the inflammatory effects are more severe in acute *C pneumoniae* infection.

5.2 Hypertensive heart disease

Hypertension is a traditional risk factor, highly prevalent and that may be severe. Arteries in patients with hypertension lose their homeostasis, promoting vasodilation in relation with the described vasoconstrictive tendency; this leads to arterial anatomic abnormalities with hyperplasia of the median layer, thus favoring the development of atherosclerosis and all the inflammatory mechanisms triggered by *C pneumoniae* that have been previously described.^{24,25}

In our study group, we found that an acute *C. pneumoniae* infection was associated with a 12-fold increase in the risk of developing hypertensive heart disease. This also suggests that acute infection may lead to more severe inflammatory mechanisms that increase the risk of CVD.

5.3 Ischemic heart disease

Its pathogenesis is closely linked to the process of atherosclerosis; there are studies that have detected viable *C pneumoniae* in the atheromatous plaque, suggesting that the bacteria triggers the inflammatory process, that in turn, can cause plaque fissures and lead to thrombotic or embolic episodes, followed by an acute coronary event.^{26,27}

Also, *C pneumoniae* DNA has been detected in peripheral blood mononuclear cells in 25.8% of patients with acute ischemic heart disease, and in 4.8% of healthy subjects. IgG was found in 73.6% of patients, and in 33.3% of controls; this also applied to IgA.²⁸

Our study showed that this group of patients had a prevalence of acute *C pneumoniae* infection of 23.08%, and a prevalence of chronic infection of 80.13 %, a value similar to that reported in the world literature.²⁹⁻³¹ In the presence of acute infection, multiple logistic regression analysis showed a

significant association, with an 18-fold increase in the possibility of having ischemic heart disease. However, when analyzing the combination of acute + chronic *C. pneumoniae* infection, we found a prevalence of 18.4%, greater than that in other groups, and statistically significant; this combination may precipitate an acute coronary syndrome due to instability of the atheromatous plaque mediated by the inflammatory processes.

5.4 Valvular heart disease

Several factors may interact in the pathogenesis of valvular sclerosis, such as oligoelements of relevance to the growth of *C. pneumoniae*, the host's defense mechanisms, and *C. pneumoniae* infection markers in sclerotic valves and in serum. In most patients with aortic sclerosis, one of several *C. pneumoniae* infection markers have been detected, and all had an altered oligoelement balance in the valves and serum, suggesting an active immune process and infection.³² An association has been established by MIF between the presence and severity of adult calcified aortic stenosis and the role of infection with *C. pneumoniae* in valvular calcification.³³

In this study, acute infection in these patients had a prevalence of 16.6%, and that of chronic infection was 68.33%. Multiple logistic regression analysis showed a 12-fold increase in the risk of having valve disease if an acute infection had been present; this coincides with reports by other authors.³⁴ Likewise, we established significance between valvular heart disease and the other forms of heart disease ($p < 0.0001$); this finding was relevant since rheumatic fever leads to valve disease, and the greatest number of patients with this history was included in this group.

In this population, we detected a prevalence of chronic infection with *C. pneumoniae* of 78.6 %, similar to that reported in the world literature.^{31, 35, 36}

5.5 Limitations

Although some of our results are consistent with different reports, we must consider that our study was conducted on a small sample, which could explain the detected protective effect of chronic infection in the development of CVD.

6. Conclusions

We observed an increased association of acute infection and CVD. The prevalence of acute + chronic infection is striking since it was higher in patients with ischemic heart disease; this suggests that the combination may precipitate an acute coronary syndrome resulting from the instability of the atheromatous plaque mediated by inflammatory processes. This is probably the first report of observations using MIF. In chronic infection, we detected a decreased risk of developing CVD. Further studies are needed to validate our findings.

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AUTHORSHIP

GGE was the principal investigator for laboratory work and assisted with data interpretation and primary manuscript writing; MFP, GRP and MMG were responsible for the biochemical data collection and analysis, and provided critical review of the manuscript; ELP contributed to data interpretation and provided critical review of the manuscript; ESM was responsible for data analyses, data interpretation and manuscript writing. All

authors have read and approved the final manuscript.

Ethical responsibilities

Protection of people

The authors declare that the procedures followed were in accordance with the ethical standards of the responsible human experimentation committee and in accordance with the World Medical Association and the Declaration of Helsinki.

Rigth to privacy and informed consent.

The participants signed letters of agreement.

Conflict of interests

The authors declare that they have no conflict of interest.

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