Chlamydia Pneumoniae Antibody Titers and Cardiac Calcifications: a Cross-Sectional Serological-Echocardiographic Correlative Study

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Abstract

Background: *Chlamydia pneumoniae* has previously been associated with higher prevalence of valvular and cardiac calcifications.

Objectives: To investigate a possible association of seropositivity for *C. pneumoniae* and the presence of cardiac calcifications (mitral annular or aortic root calcification, and aortic valve sclerosis).

Methods: We retrospectively analyzed serological data (immunoglobulin G TWAR antibodies) from the AZACS trial (**Az**ithromycin in **A**cute **C**oronary **S**yndromes), and correlated the serological findings according to titer levels with the presence of cardiac calcifications as detected by transthoracic echocardiography.

Results: In 271 patients, age 69 ± 13 years, who underwent both serological and echocardiographic evaluation, we found no significant association between the "calcification sum score" (on a scale of 0–3) in seropositive compared to seronegative patients (1.56 ± 1.15 vs.1.35 ± 1.15, respectively, $P = 0.26$). The median calcification sum score was 1 (interquartile range 0–3) for the seronegative group, and 2 (interquartile range 0–3) for the seropositive group (P = 0.2757). In addition, we did not find a significant correlation of any of the individual sites of cardiac calcification and *C. pneumoniae* seropositivity.

Conclusion: Our findings suggest that past *C. pneumoniae* infection may not be associated with the pathogenesis of valvular and cardiac calcifications.

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Chlamydia pneumoniae, a common respiratory pathogen, is frequently detected in pathological specimens of atherosclerotic plaques, and has been suggested as a possible cause of coronary artery disease [1]. The current body of evidence establishes this pathogen as a plausible, potentially modifiable risk factor in cardiovascular disease [2]. Strong associations exist between C. *pneumoniae* infection and atherosclerosis, as demonstrated by: a) sero-epidemiological studies showing that patients with cardiovascular disease have higher titers of anti-C. *pneumoniae* antibodies compared with control patients; b) pathological studies detecting the organism within atherosclerotic lesions but not in adjacent normal tissue, by immunohistochemistry, polymerase chain reaction and electron microscopy and by culturing the organism from lesions; and c) laboratory studies showing that C. *pneumoniae* can either initiate lesion development or cause exacerbation of lesions in rabbit and mouse animal models respectively [3]. Studies of C. *pneumoniae* pathogenesis have shown that the organism can infect many cell types associated with both respiratory and cardiovascular sites, including lung epithelium and resident alveolar macrophages, circulating monocytes, arterial smooth muscle cells and vascular endothelium [3,4]. Infected cells have been shown to exhibit characteristics associated with the development of cardiovascular disease (e.g., secretion of pro-inflammatory cytokines and procoagulants by infected endothelial cells and foam cell formation by infected macrophages) [3,5,6].

Cardiac calcifications (mitral annular calcification, aortic root calcification and aortic valve sclerosis) were recently found to be associated with a higher prevalence and severity of atherosclerotic vascular lesions [7-9]. Additional studies have suggested that C. *pneumoniae* may be involved in the pathogenesis of degenerative aortic valve disease [10,11], whereas other studies failed to show such an association [4,12]. However, the immunological and immunochemistry findings in previous studies found a very high prevalence (> 80%) of past C. *pneumoniae* infection in the adult population, irrespective of heart valve disease [4].

The AZACS study (**Az**ithromycin in **A**cute **C**oronary **S**yndromes) was a multi-center randomized double-blind placebo-controlled study that tested the effect of short-term anti-C. *pneumoniae* antibiotic treatment on recurrent ischemic events [13]. The results over 6 months show that an early 5 day course of azithromycin after an acute coronary syndrome did not reduce the number of patients who died or developed myocardial infarction, or the number of ischemic episodes requiring percutaneous coronary intervention or coronary artery bypass graft [13]. Similarly, the frequency of recurrent ischemia or congestive heart failure necessitating admission was also not affected by treatment with azithromycin. Furthermore, treatment with azithromycin did not reduce adverse clinical outcomes in patients who tested positive for C. *pneumoniae* [13].

In view of the recent conflicting data on the effect of C. *pneumoniae* on cardiac calcifications and its association with cardiovascular events, we decided to analyze the AZACS study data for a possible association of C. *pneumoniae* seropositivity with echocardiographically detected cardiac calcifications.

Patients and Methods

The AZACS trial assessed the effect of azithromycin in a multicenter double-blind randomized trial in 1439 patients with unstable angina or acute myocardial infarction. Patients were randomly allocated to receive 500 mg azithromycin on the first day after randomization, followed by 250 mg daily or placebo for

4 days. Patients were followed for 6 months. The primary endpoints were death, recurrent myocardial infarction, or recurrent ischemia necessitating revascularization. Patients were excluded if they had Q-wave myocardial infarction within the past 28 days of the qualifying admission, allergy to any macrolide antibiotic, or any significant diseases that could compromise the patient's safety or participation in the study. Analysis was done by intention to treat.

In this study we evaluated all the patients randomized in the AZACS trial at Cedars-Sinai Medical Center who underwent transthoracic echocardiographic studies during the index hospitalization. The transthoracic echocardiographic studies were interpreted by one of two expert readers for the presence of: a) mitral annular calcification – defined as an intense echo-producing structure located at the junction of the atrioventricular groove and posterior mitral valve leaflet on the parasternal long axis, apical four-chamber, or parasternal short-axis views; b) mitral annular calcification; and c) aortic valve sclerosis. Calcium deposits in the aortic valve and aortic root were defined by focal area of increased echogenicity and thickening. The extent of calcification was scored according to the number of sites of calcium deposits in mitral annulus, aortic valve and aortic root (sum = 0–3).

Baseline C. *pneumoniae* (TWAR antibodies TW 183) immunoglobulin G antibodies were tested by micro-immunofluorescence (MRL, Focus Technologies, Cypress, CA, USA). Seropositivity was defined as an IgG titer of ≥ 1:16 and seronegativity was defined as IgG titer < 1:16 [13]. Among seropositive subjects, antibody titers were defined as low (1:16 to 1: < 128), intermediate (1:128 to 1:256), or high (> 1:256). We did not study the correlation of cardiac calcifications with IgA or lipopolysaccharide.

Statistical analysis

Data are presented as means ± SD and as medians and interquartiles. The Pearson chi-square test was used for categorical variables and the Mann-Whitney test was used to evaluate for significant differences in medians between the groups for a possible association of cardiac calcifications with C. *pneumoniae* IgG titers. We considered $P < 0.05$ as statistically significant.

Results

The AZACS study randomized 1439 patients, of whom 1412 completed the follow-up period. Of those, 939 patients (84%) were found positive for past or recent C. *pneumoniae* infection by IgG seropositivity. We analyzed the echocardiographic studies of 271 patients, age 69 ± 13 years, recruited in a single hospital (Cedars-Sinai Medical Center) who underwent transthoracic echocardiography during hospitalization: 223 patients were seropositive for C. *pneumoniae* and 48 patients were seronegative.

The patients' baseline characteristics are presented in Table 1. The baseline characteristics of our patients did not differ in any parameter from the entire cohort of patients recruited in the AZACS trial. The mean age of patients who were seropositive for C. *pneumoniae* was higher than in those who were seronegative.

IgG = immunoglobulin G

Table 1. Patients' baseline characteristics

	IgG positive for C. *pneumoniae* (n=223)	IgG negative for C. *pneumoniae* (n=48)
Age (yrs)*	70 ± 13	67 ± 14
Male gender (%)	74	72
Diabetes (%)	27	28
Hypertension (%)	58	57
Hyperlipidemia (%)	59	61
Smoking (%)	52	52
Family history of coronary artery disease (%)	28	26
Statins (%)	62	62

*$P = 0.09$

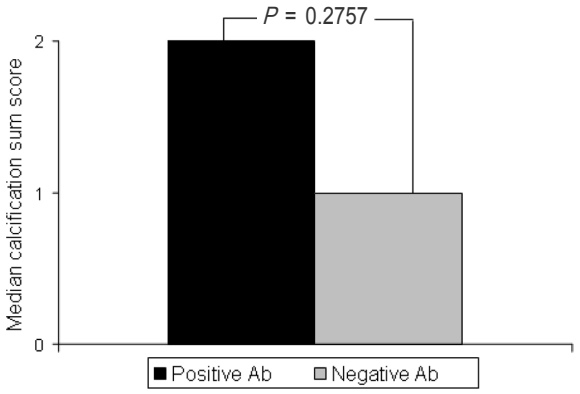

Figure 1. Median calcification sum score in *C. pneumoniae* seropositive compared to seronegative patients.

Otherwise, there were no significant differences between the groups. Statins and lipid-lowering drugs were equally used in both the C. *pneumoniae* seropositive and seronegative groups both at baseline and at 6 month follow-up, respectively (62% and 62%, 73% and 69%).

As presented in Figure 1, the median calcification sum score was 1 (interquartile range 0–3) for the seronegative group, and 2 (interquartile range 0–3) for the seropositive group ($P = 0.2757$). The mean calcification sum score was higher in the C. *pneumoniae*-seropositive group compared to the seronegative group; however, this difference also did not reach statistical significance (1.56 ± 1.15 vs. 1.35 ± 1.15, respectively, $P = 0.26$). We did not find a significant association of C. *pneumoniae* antibody seropositivity with any of the individual components of the calcification sum score – patients with or without mitral annular calcification (80% vs. 85%, respectively, $P = 0.273$), with or without aortic root calcification (81% vs. 83%, respectively, $P = 0.640$), and with or without aortic valve sclerosis (79% vs. 85%, respectively, $P = 0.251$). As presented in Table 2, there was no significant correlation between the IgG titer and the calcification sum score or any of its individual components. In patients older than 70 years, there was a trend ($P = 0.09$) for an association of C. *pneumoniae* seropositivity with the calcification sum score compared to younger patients.

Table 2. Association of *C. pneumoniae* IgG antibody titer with site and extent of calcification

	IgG titer				
	<1:16 (n=48)	1:16–<1:128 (n=88)	1:128–1:256 (n=96)	1:>256 (n=39)	*P*
Mitral annular calcification (%)					0.726
Present	20	32	33	14	
Absent	15	33	38	15	
Aortic valve sclerosis (%)					0.284
Present	21	29	32	17	
Absent	15	35	37	12	
Aortic root calcification (%)					0.697
Present	19	29	38	14	
Absent	17	35	33	15	
Total (%)					0.899
Score=0	21	31	32	16	
Score=3	14	37	33	16	

Discussion

The results of the study suggest that IgG seropositivity for C. *pneumoniae* is not associated with a higher prevalence of cardiac calcifications, and that the pathogenesis of cardiac calcifications may not be related to previous C. *pneumoniae* infection. Although previous studies have shown a possible association of atherosclerosis with cardiac calcifications, as well as an association between previous C. *pneumoniae* infection and atherosclerosis, we could not find such an association [10,11,14-17]. A recently published study by Turgeman et al. [18] suggested an association of past C. *pneumoniae* infection with severity of calcific aortic stenosis. However, their study was relatively small, and the effect of C. *pneumoniae* infection on the prevalence of aortic valve calcification could not be isolated due to the significantly greater use of drugs with anti-inflammatory properties (e.g., aspirin, statins and angiotensin-converting enzyme inhibitors). Moreover, since valvular calcification is age-related [7], Turgeman et al. [18] found a significantly lower aortic valve area in older patients.

Our findings are in accordance with previously published serologic-echocardiographic correlative studies that did not demonstrate an association of cardiac calcifications with C. *pneumoniae* seropositivity [19,20]. In addition, several recently published studies also failed to show a protective effect of anti-C. *pneumoniae* antibiotic therapy in reducing coronary events in patients with acute coronary syndromes [21,22]. The results of these studies have reduced the likelihood that C. *pneumoniae* is associated with the pathogenesis or the progression of atherosclerosis or the occurrence of an acute coronary syndrome.

Our study is a sub-analysis of a well-designed randomized placebo-controlled large trial [13]. The patients included in the study had transthoracic echocardiography in close time proximity to their admission with acute coronary syndrome. This increases the validity of our results. We did not study a possible association of IgA C. *pneumoniae* antibodies with cardiac calcifications since the formation of calcifications is a chronic process rather than an acute reaction to infection, inflammation or any other type of insult.

Study limitations

The study population consisted of patients with acute coronary syndrome who were shown to have increased titers of C. *pneumoniae* [23] and may not be 'real control' patients even if they do not have valvular calcifications. Therefore, the results of our study should be interpreted with caution and may be relevant only to acute coronary syndrome patients. The findings could be entirely different in a different group of patients with a lower prevalence of C. *pneumoniae* infection. The sensitivity of transthoracic echocardiography to detect valvular or aortic calcification may be limited. Each transthoracic echocardiographic study was interpreted by a single reader, and may thus have been subject to misinterpretation. The study is cross-sectional and therefore cannot address the question if high titers of C. *pneumoniae* antibodies predict later development of cardiac calcifications. Serology is unable to diagnose the presence of C. *pneumoniae* bacteria in the valvular calcific lesions, indicating the true effect of C. *pneumoniae* on the development of valvular calcifications [24]. In older people the incidence of positive serology for C. *pneumoniae* is high and does not leave much room to show increased titers. Since antibodies for C. *pneumoniae* may last for 3–5 years, even negative patients may have come into contact with this bacterium. These factors and others make interpretations and conclusions based on C. *pneumoniae* serology difficult.

In conclusion, we did not find an association of cardiac calcifications with IgG seropositivity to be a marker of previous C. *pneumoniae* infection. These findings suggest that this pathogen may not be associated with the pathogenesis of cardiac calcifications.

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