Adult Calcific Aortic Stenosis and *Chlamydia pneumoniae*: the Role of Chlamydia Infection in Valvular Calcification

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Abstract

Background: Adult calcific aortic stenosis is a well-known clinical entity but its pathophysiology and cellular mechanism have yet to be defined.

Objectives: To determine whether there is an association between the presence and severity of adult calcific aortic stenosis and *Chlamydia pneumoniae* seropositivity

Methods: Forty adult patients (23 women, 17 men) were divided into three groups according to echocardiographic aortic valve area: Group A – 7 symptomatic subjects (age 67 ± 7 years) with normal aortic valve and normal coronary angiogram, Group B – 16 patients (age 73 ± 6) with moderate ACAS (AVA > 0.8 ≤ 1.5 cm^2), and Group C – 17 patients (age 76 ± 7) with severe ACAS (AVA ≤ 0.8 cm^2). We tested for immunoglobulins M, G and A as retrospective evidence of *C. pneumoniae* infection using the micro-immunofluorescence method. Past *C. pneumoniae* infection was defined by IgG titer > 16 ≤ 512.

Results: No patients in group A showed positive Ig for *C. pneumoniae*. IgM was not detected in any of the patients with ACAS (groups B and C) while 2 of 17 patients (12%) in group C showed IgA for the pathogen. High titers of IgG were found in 14 of 33 (42%) of the patients with moderate or severe ACAS: 5 of 16 (31%) in group B and 9 of 17 (53%) in group C ($P = 0.2$). Both groups had the same prevalence of coronary artery disease (66%). AVA was lower in IgG-seropositive patients than in the seronegative group (0.88 ± 0.3 cm^2 vs. 1.22 ± 0.4 cm^2, respectively, $P = 0.02$).

Conclusions: Past *C. pneumoniae* infection may be associated with a higher prevalence and greater severity of ACAS.

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Adult calcific aortic stenosis is the most frequent reason for aortic valve replacement in western countries [1]. This condition increases in prevalence with advancing age, affecting 2–3% of the adult population over the age of 65 [2]. Although the macroscopic pathology of this clinical entity was described more than 150 years ago [3], the pathophysiologic mechanisms including the cellular basis of this valvular abnormality have never been elucidated.

ACAS = adult calcific aortic stenosis
AVA = aortic valve area
Ig = immunoglobulin

Recently, animal studies supported the hypothesis that ACAS may be a consequence of an inflammatory process similar to the etiology of atherosclerosis, with the concept of 'response to injury' serving as the main mechanism for both conditions [4]. Injury to the endothelium by different triggers is the key event behind this concept. Several forms of endothelial damage caused by different agents have been described, including infection by microorganisms such as viruses and bacteria [5]. *Chlamydia pneumoniae* is a common cause of respiratory infections worldwide. Epidemiologic studies have pointed to an association between atherosclerosis and C. *pneumoniae* infection and this pathogen has been found in a variety of atherosclerotic lesions [6]. The aim of our study was first to clarify the role of C. *pneumoniae* as an infectious trigger for the development of valvular ACAS and, second, to explore a possible association between immunoglobulin level for this bacterium and the severity of valvular stenosis.

Patients and Methods

Study population

Based on transthoracic echocardiographic aortic valve area calculated by the continuity equation [7], we enrolled 40 adult patients: 23 females and 17 males with a mean age of 74 ± 7 years (range 60–94). The patients were divided into three groups: 7 subjects with chest pain but normal aortic valve morphology and function and normal coronary angiography (group A), 16 patients with moderate aortic stenosis (AVA > 0.8 ≤ 1.5 cm^2) (group B), and 17 patients with severe ACAS [8] (AVA ≤ 0.8 cm^2) (group C).

Data collection

AVA (cm^2) as determined by two-dimensional transthoracic echocardiography and peak instantaneous gradient (mmHg) were the initial data collected. Medical records from the valvular outpatient clinic including coronary angiographic reports were used as a data source for patients' age, gender, presence of major risk factors for atherosclerosis, coronary angiographic status and extracardiac disorders, including actual medical therapy. The presence of significant coronary artery disease was determined by angiography and defined by at least one major epicardial vessel having a narrowing of > 70% by visual estimation.

At the end of the echocardiographic evaluation, blood was

taken for specific antibodies to C. *pneumoniae*, and for measuring immunoglobulins G, M and A, erythrocyte sedimentation rate, C-reactive protein, creatinine, calcium, phosphorus, total cholesterol, triglyceride and low density lipoprotein. Serologic evaluation was performed by means of the micro-immunofluorescence test (MIF MRL Diagnostics, Cypress, CA, USA). Past or preexisting infection was defined on the basis of an IgG titer between 16 and 512 [9].

Patients less than 60 years old with aortic stenosis or those with a bicuspid valve revealed by echocardiography were excluded from the study. Patients with recent myocardial infarction were also excluded since IgG titer can be elevated due to the appearance of antibodies against heat shock protein, a phenomenon described by Hoppichler et al. [10]. We also excluded patients with ACAS who suffered from an associated disease such as acute febrile illness, chronic inflammatory disease, malignancy, or hematologic disorder, or those with renal failure (serum creatinine > 2 mg/dl), since these may affect ESR or CRP or may add an additional factor that may interfere with the process of valvular calcification.

Table 1. Echocardiographic, coronary angiographic findings and clinical characteristics of study patients

Echocardography/ Angiography	Group A (n=7)	Group B (n=16)	Group C (n=17)	*P*
AVA (cm^2)	1.9 ± 0.2	1.3 ± 0.2	0.7 ± 0.1	<0.0001
PI-Gr (mmHg)	5 ± 4	21 ± 7	54 ± 25	<0.0001
CAD (%)	Ø	64	64	0.009
Age (yrs)	66 ± 6	73 ± 2	76 ± 7	0.01
NYHA II/III	4/3	7/9	6/11	NS
Smoking (%)	29	25	24	NS
Hypertension (%)	100	63	71	NS
Diabetes mellitus (%)	29	19	18	NS
Dyslipidemia (%)	29	69	53	NS
Beta-blockers (%)	57	63	47	NS
Calcium blockers (%)	29	38	24	NS
ACE-inhibitors (%)	57	31	18	NS
Aspirin (%)	86	63	47	NS
Statins (%)	71	50	41	NS

PI-Gr = peak instantaneous gradient, CAD = coronary artery disease, NYHA =- New York Heart Association class. Ø =none.

Statistical analysis

Data analysis was performed using the SPSS 11.5 statistical package. The association between the categorical variables was examined using the Fisher exact test for small groups. Comparison of continuous variables between the three study groups was analyzed by ANOVA or the Kruskal-Wallis test when appropriate. The *t*-test was performed to compare continuous variables between two independent groups. Pearson correlation coefficients were calculated. To assess the influence of different variables on the AVA level, a multiple linear regression model was used.

Results

Patients' baseline echocardiographic, angiographic and clinical characteristics are presented in Table 1. The patients from group C were older than those of the other groups: mean age 76 ± 7 years compared with 67 ± 7 and 73 ± 6 in groups A and B respectively ($P = 0.01$) .

The mean AVA and mean peak instantaneous gradient across the aortic valve of patients of group C were 0.7 ± 0.1 cm^2 and 54 ± 25 mmHg respectively. These parameters in group B were 1.3 ± 0.2 cm^2 and 21 ± 7 mmHg respectively. Aortic valve area and peak instantaneous gradient across aortic valve in group A were in the normal range. None of the patients in group A presented with coronary atherosclerosis upon examination by angiography whereas two thirds of the patients in groups B and C showed significant coronary artery disease.

There was no statistically significant difference between the groups regarding major risk factors for atherosclerosis. All patients in group A had hypertension but only four (57%) were treated by either beta-blockers or angiotensin-converting enzyme inhibitors. In group C, 12 patients (71%) had hypertension, however only 3 (18%) were treated with ACE inhibitors. Six patients in group A (86%) received aspirin, compared to only 8 in group C (47%). In group C, 9 patients (53%) had dyslipidema but only 7 (41%) received statins, while in group A only 2 patients (29%) had dyslipidemia and 5 patients (71%) were treated with statins.

Chemical laboratory data [Table 2]

Mean creatinine level was significantly higher in group C than in group A: 1.2 ± 0.2 versus 0.9 ± 0.1 mg/dl respectively ($P = 0.023$). No significant differences for other chemical parameters were found between the groups.

Association of concomitant drug therapy and AVA [Table 3]

Although not statistically significant, mean AVA was higher among patients treated by either beta-blockers or calcium antagonists compared to the non-treated group: 1.2 ± 0.4 vs. 1 ± 0.4 cm^2 respectively. However, statistically significant higher mean AVA values were found among patients treated by either ACE-inhibitors (captopril 5%, enalapril 75%, ramipril 20%), aspirin (acetylsalicylic acid with a variety of dosages, 75–325 mg/day) or statins (simvastatin 90% and atorvastatin 10%) compared to the non-treated group: 1.3 ± 0.4 vs. 1 ± 0.4 cm^2 ($P = 0.038$) for ACE inhibitors, 1.2 ± 0.4 vs. 0.9 ± 0.4 cm^2 for aspirin ($P = 0.033$) and 1.3 ± 0.4 vs. 0.9 ± 0.3 cm^2 for statins ($P = 0.054$)

Immunoserologic evaluation [Table 4]

No C. *pneumoniae* immunoglobulin of any type was detected in serologic testing of group A. Moreover, no IgM for C. *pneumoniae* was found in any of the patients in groups B and C. IgA for this pathogen was found in only 2 of the 17 patients (12%) in group

ESR = erythrocyte sedimentation rate
CRP = C-reactive protein

ACE = angiotensin-converting enzyme

Table 2. Chemical laboratory data of study population

CLD	Group A (n=7)	Group B (n=16)	Group C (n=17)	*P*
Creatinine (mg/dl)	0.9 ± 0.1	1.0 ± 0.2	1.2 ± 0.2	0.023
Range	8.8–9.5	0.8–1.7	0.9–1.8	
Calcium (mg/dl)	9.1 ± 0.2	8.9 ± 0.5	8.8 ± 0.5	0.52
Range	8.8–9.5	7.9–9.6	7.5–9.5	
Phosphorus (mg/dl)	3.7 ± 0.7	3.6 ± 0.6	3.5 ± 0.7	0.81
Range	2.8–5.2	3–5.6	2.2–5.3	
Total cholesterol (mg/dl)	186 ± 35	190 ± 37	185 ± 50	0.91
Range	123–222	116–225	106–258	
Triglycerides (mg/dl)	166 ±57	180 ± 64	102 ± 24	0.16
Range	92–263	67–262	76–264	
Low density lipoprotein (mg/dl)	115 ± 20	125 ± 15	135 ± 17	0.24
Range	96–155	90–135	99–156	
ESR (mm/hr)	21 ± 20	41 ± 29	30 ± 17	0.11
Range	5–61	12–54	2–62	
CRP (mg/L)	7 ± 3	10 ± 8	17 ± 14	0.02

Table 3. The association of concomitant drug therapy and aortic valve area

Drug	No treatment AVA (cm^2)	Treatment AVA (cm^2)	*P*
Beta-blockers	n=18	n=22	0.2
	1 ± 0.4	1.2 ± 0.4	
Calcium antagonists	n=28	n=12	0.5
	1 ± 0.4	1.2 ± 0.4	
ACE-inhibitors	n=26	n=12	0.038
	1 ± 0.4	1.3 ± 0.4	
Aspirin	n=16	n=24	0.033
	0.9 ± 0.4	1.2 ± 0.4	
Statins	n=19	n=21	0.054
	0.9 ± 0.3	1.3 ± 0.4	

C. However, 5 of 16 (31%) patients in group B and 9 of 17 (53%) in group C had IgG for C. *pneumoniae* ($P = 0.55$).

We could not establish a statistically significant correlation between low density lipoprotein level or statin therapy and the presence of IgG for C. *pneumoniae*. Among the patients treated with statins, 30% were found to have IgG for this pathogen, compared to 42% among those not treated with statins ($P = 0.43$).

Among the 33 patients with either moderate (group B) or severe ACAS (group C), mean AVA was 1.22 ± 0.2 cm^2 in the 19 seronegative patients and 0.88 ± 0.5 cm^2 in the 14 seropositive patients ($P = 0.02$). Quantitative evaluation of IgG in groups B and C showed that a titer of 1/512 for C. *pneumoniae* was detected only in group C patients.

Regression models

Multiple predictors for ACAS were found by using univariate analysis: age ($P = 0.016$), creatinine ($P = 0.021$), and the presence of IgG to C. *pneumoniae* ($P = 0.02$). Treating patients with ACE inhibitors, aspirin or statins was correlated with higher AVA as compared to the non-treated groups [Table 3]. There was no correlation between smoking, cholesterol level, treatment with either beta-blockers or calcium antagonists and the presence of ACAS. However, a weak correlation was found between the presence of coronary artery disease and ACAS ($P = 0.092$).

Table 4. Incidence of immunoglobulin for C. *pneumoniae* and IgG count

	Group A (n=7)	Group B (n=16)	Group C (n=17)
Immunoglobulins			
IgM	Ø	Ø	Ø
IgA	Ø	Ø	2/17 (12%)
IgG	Ø	5/16 (31%)	9/17 (53%) $P = 0.055$
IgG count			
0	7 (100%)	11 (69%)	8 (47%)
1/64	Ø	1 (7%)	2 (12%)
1/128	Ø	2 (12%)	2 (12%)
1/256	Ø	2 (12%)	3 (17%)
1/512	Ø	Ø	2 (12%)

By using multivariate analysis, a linear correlation was found between AVA and both creatinine level and the presence of IgG to C. *pneumoniae*: $y = 1.976–0.367$, IgG 0.652, creatinine, $R^2 = 0.268$, $F = 6.8$ ($P = 0.004$).

Discussion

Our data showed that the presence of IgG to C. *pneumoniae*, aging, and high serum creatinine level even in the normal range has a negative impact on AVA, whereas therapy with aspirin, statins and ACE-inhibitors has some protective effect against progression of ACAS.

Many reports have provided evidence that ACAS has much in common with atherosclerosis. Both are active and dynamic processes often beginning in early adulthood [11]. They share a similar cellular pathophysiology involving multiple physical, chemical and infectious triggers that result in vascular wall abnormalities [12]. Recent studies have indicated that arresting the progress of these vascular wall pathologies can be achieved by the same group of medications [13].

Our study confirms previous observations [14] that aging is directly related to the severity of ACAS. The most narrowed AVA were found among the oldest patients studied. In recognition of the fact that either degenerative or calcific aortic stenosis may also appear in relatively young people, we accept the terminology of Rahimtoola [15]: namely, that among elderly people this valvular pathology should be termed "age related aortic stenosis" instead of simply adult calcific aortic stenosis.

Previous data have shown that hemodialysis or abnormally high creatinine levels are associated with rapid progression of ACAS [16]. In our study, although we excluded patients with renal failure we demonstrated that even within the normal range, a higher creatinine level is associated with lower AVA.

In a manner similar to the etiology of atherosclerosis, active inflammatory components leading to bone formation have been described as part of the pathogenesis of calcific aortic valve

disease [17]. This observation is strengthened indirectly by our finding of higher AVA values among patients treated with various drugs (such as aspirin, statins and ACE-inhibitors) that may have anti-inflammatory effects [18–20].

The first serologic evidence of an association between the obligate intracellular bacterium C. *pneumoniae* and atherosclerosis of the coronary arteries was discovered in 1988 [21]. Following this, several studies suggested a relationship between the presence of IgG to C. *pneumoniae* and ACAS. Juvonen et al. [22] found C. *pneumoniae* to be frequently present in aortic valve tissue and associated with early lesions of aortic stenosis among the elderly. Our results lend support to the theory that C. *pneumoniae* as an infectious agent plays some role in this vascular wall pathology. Although the numbers of our sample groups are small, it is noteworthy that IgG to C. *pneumoniae* was not detected among the group characterized by the absence of coronary atherosclerosis and exhibiting normal aortic valves. On the other hand, among patients with ACAS the group seropositive for C. *pneumoniae* had lower AVA values than the seronegative group. From a quantitative point of view, even though statistically not significant, an IgG titer of 512 was detected only in group C, characterized by AVA $\leq$ 0.8 cm^2. Although endothelial function tests were not performed in this study, it seems that a correlation between the C. *pneumoniae* titers and endothelial function assessment would have elucidated this basic cellular pathophysiology.

However, in attempting to explain the mechanism underlying these observations, two possibilities should be examined. The first is whether initial endothelial injury caused by C. *pneumoniae* induces the atherosclerotic process, or alternatively, does the presence of this organism in the cellular milieu accelerate the progression or severity of the disease. Based on animal studies and *in vitro* evaluation it seems that both mechanisms coexist. C. *pneumoniae* reproduces in human macrophages, endothelial cells and arterial smooth muscle cells – all key cellular components in atherosclerosis [23]. This infection of human vascular endothelial cells results in production of cytokines, leading to activation of monocytes, platelets and macrophages [24]. These, in turn, promote osteoblast-like activity and matrix synthesis and most probably contribute to valvular calcification and ossification [4].

Multiple questions regarding the issue of ACAS and C. *pneumoniae* still remain. Also unknown is the number of people in the general population with ACAS and high C. *pneumoniae* titers, compared to those with normal C. *pneumoniae* titers. What is needed is a comparison on progress; that is, the high versus normal titers of patients with ACAS and the same AVA should be monitored prospectively. This will undoubtedly contribute to our knowledge about this type of process. What is the meaning of high C. *pneumoniae* titers in these patients? Atherosclerosis and ACAS share many similarities, yet the lack of success of anti-C. *pneumoniae* therapy in large prospective double-blind clinical trials in coronary artery disease patients [25] leaves this question unresolved. Hopefully, extending our understanding of these cellular processes will yield improved therapeutic tools in the future.

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