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

Yoav Turgeman MD, Pierre Levahar MD, Idit Lavi MA, Amir Shneor PhD, Raoul Colodner PhD, Zmira Samra PhD, Lev Bloch MD and Tiberio Rosenfeld MD

1Department of Cardiology and 2Clinical Laboratories, HaEmek Medical Center, Afula, Israel
3Department of Community Medicine and Epidemiology, Carmel Medical Center, Haifa, Israel
4Microbiology Laboratory, Rabin Medical Center (Beilinson Campus), Petah Tiqva, Israel

Key words: adult calcific aortic stenosis, *Chlamydia pneumoniae*, immunoglobulins, aging.

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 cm²), and Group C – 17 patients (age 76 ± 7) with severe ACAS (AVA ≤ 0.8 cm²). 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 cm² vs. 1.22 ± 0.4 cm², respectively, P = 0.02).

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

IMAJ 2006;8:464–468

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.

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 cm²) (group B), and 17 patients with severe ACAS [8] (AVA ≤ 0.8 cm²) (group C).

Data collection

AVA (cm²) 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.

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 cm² and 54 ± 25 mmHg respectively. These parameters in group B were 1.3 ± 0.2 cm² 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 dyslipidemia 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 cm² 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 cm² (P = 0.038) for ACE inhibitors, 1.2 ± 0.4 vs. 0.9 ± 0.4 cm² for aspirin (P = 0.033) and 1.3 ± 0.4 vs. 0.9 ± 0.3 cm² 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

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

<table>
<thead>
<tr>
<th>Echocardiography/ Angiography</th>
<th>Group A (n=17)</th>
<th>Group B (n=18)</th>
<th>Group C (n=17)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVA (cm²)</td>
<td>1.9 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PI-Gr (mmHg)</td>
<td>5 ± 4</td>
<td>21 ± 7</td>
<td>54 ± 25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CAD (%)</td>
<td>64</td>
<td>64</td>
<td>64</td>
<td>0.009</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>66 ± 6</td>
<td>73 ± 2</td>
<td>76 ± 7</td>
<td>0.01</td>
</tr>
<tr>
<td>NYHA II/III</td>
<td>4/3</td>
<td>7/9</td>
<td>6/11</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>29</td>
<td>25</td>
<td>24</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>100</td>
<td>63</td>
<td>71</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>29</td>
<td>19</td>
<td>18</td>
<td>NS</td>
</tr>
<tr>
<td>Dyslipidemia (%)</td>
<td>29</td>
<td>69</td>
<td>53</td>
<td>NS</td>
</tr>
<tr>
<td>Beta-blockers (%)</td>
<td>57</td>
<td>63</td>
<td>47</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium blockers (%)</td>
<td>29</td>
<td>38</td>
<td>24</td>
<td>NS</td>
</tr>
<tr>
<td>ACE-inhibitors (%)</td>
<td>57</td>
<td>31</td>
<td>18</td>
<td>NS</td>
</tr>
<tr>
<td>Aspirin (%)</td>
<td>86</td>
<td>63</td>
<td>47</td>
<td>NS</td>
</tr>
<tr>
<td>Statins (%)</td>
<td>71</td>
<td>50</td>
<td>41</td>
<td>NS</td>
</tr>
</tbody>
</table>

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

ESR = erythrocyte sedimentation rate
CRP = C-reactive protein

ACE = angiotensin-converting enzyme
Table 2. Chemical laboratory data of study population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A (n=7)</th>
<th>Group B (n=16)</th>
<th>Group C (n=17)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>0.023</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>8.8–9.5</td>
<td>8.6–1.7</td>
<td>9.0–1.9</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>9.1 ± 0.2</td>
<td>8.9 ± 0.5</td>
<td>8.8 ± 0.5</td>
<td>0.52</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>8.8–9.5</td>
<td>7.9–9.6</td>
<td>7.5–9.5</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>3.7 ± 0.7</td>
<td>3.6 ± 0.6</td>
<td>3.5 ± 0.7</td>
<td>0.81</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>186 ± 35</td>
<td>190 ± 37</td>
<td>185 ± 50</td>
<td>0.91</td>
</tr>
<tr>
<td>Range</td>
<td>123–222</td>
<td>116–225</td>
<td>106–258</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>166 ± 57</td>
<td>180 ± 64</td>
<td>102 ± 24</td>
<td>0.16</td>
</tr>
<tr>
<td>Range</td>
<td>92–203</td>
<td>67–202</td>
<td>70–264</td>
<td></td>
</tr>
<tr>
<td>Low density lipoprotein (mg/dl)</td>
<td>115 ± 20</td>
<td>125 ± 15</td>
<td>135 ± 17</td>
<td>0.24</td>
</tr>
<tr>
<td>Range</td>
<td>96–155</td>
<td>90–153</td>
<td>99–156</td>
<td></td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>21 ± 20</td>
<td>41 ± 29</td>
<td>30 ± 17</td>
<td>0.11</td>
</tr>
<tr>
<td>Range</td>
<td>5–61</td>
<td>12–54</td>
<td>2–62</td>
<td></td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>7 ± 3</td>
<td>10 ± 8</td>
<td>17 ± 14</td>
<td>0.02</td>
</tr>
</tbody>
</table>

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 cm² in the 19 seronegative patients and 0.88 ± 0.5 cm² 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).

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, R² = 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.
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. Juvenonen 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 ≤ 0.8 cm². 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.

References


Correspondence: Dr. Y. Turgeman, Director of Invasive Cardiology, Dept. of Cardiology, HaEmek Medical Center, Afula 18101, Israel.
Phone: (972-4) 649-4016
Fax: (972-4) 649-4387
email: yoav_t@clalit.org.il

In a consumer society there are inevitably two kinds of slaves: the prisoners of addiction and the prisoners of envy
Ivan Illich (1926-2002), Austrian-born U.S. priest, educator and writer

Capsule

Alcohol drinking patterns have different CHD outcomes in men and women

The inverse association between drinking alcohol and risk of coronary heart disease (CHD) seems to be independent of drinking frequency in women but not in men. Tolstrup and colleagues looked at alcohol drinking patterns in a cohort of more than 50,000 middle-aged women and men free of cardiovascular disease at baseline. After a median follow-up of 6 years, women who drank alcohol on at least one day a week had a reduced risk of CHD compared with those who drank less often, but above this frequency intake mattered more than frequency. Among men, frequency mattered more than intake, with the lowest risk in those who drank daily.

Br Med J 2006;332:1244
Eitan Israeli

Capsule

Citation advantage of open-access articles

Gunther Eysenbach from the University of Toronto assessed the potential of open-access articles. Open access (OA) to the research literature has the potential to accelerate recognition and dissemination of research findings, but its actual effects are controversial. This was a longitudinal bibliometric analysis of a cohort of OA and non-OA articles published between 8 June and 20 December 2004 in the same journal. Article characteristics were extracted, and citation data were compared between the two groups at three different time points: at “quasi-baseline” (December 2004, 0–6 months after publication), in April 2005 (4–10 months after publication), and in October 2005 (10–16 months after publication). Potentially confounding variables – including number of authors, authors’ lifetime publication count and impact, submission track, country of corresponding author, funding organization, and discipline – were adjusted for in logistic and linear multiple regression models. A total of 1492 original research articles were analyzed: 212 (14.2% of all articles) were OA articles paid by the author, and 1280 (85.8%) were non-OA articles. In April 2005 (mean 206 days after publication), 627 (49.0%) of the non-OA articles versus 78 (36.8%) of the OA articles were not cited. Six months later (mean 288 days after publication), non-OA articles were still more likely to be uncited (non-OA: 172 [13.6%], OA: 11 [5.2%]). The average number of citations of OA articles was higher compared to non-OA articles (April 2005: 1.5 versus 4.5). In a logistic regression model, controlling for potential confounders, OA articles compared to non-OA articles remained twice as likely to be cited (odds ratio = 2.1 [1.5–2.9]) in the first 4–10 months after publication (April 2005), with the odds ratio increasing to 2.9 (1.5–5.5) 10–16 months after publication (October 2005). Articles published as an immediate OA article on the journal site have higher impact than self-archived or otherwise openly accessible OA articles. The author found strong evidence that, even in a journal that is widely available in research libraries, OA articles are more immediately recognized and cited by peers than non-OA articles published in the same journal. OA is likely to benefit science by accelerating dissemination and uptake of research findings.

PloS Biol 2006;4 (May)
Eitan Israeli