Invasive measurements of pulse wave velocity correlate with the degree of aortic valve calcification and severity associated with matrix metalloproteinases in elderly patients with aortic valve stenosis

Ping-Yen LIU*, Wei-Chuan TSAI*, Chih-Chan LIN‡, Chih-Hsin HSU*, Yao-Yi HAUNG‡ and Jyh-Hong CHEN*

*Division of Cardiology, Department of Internal Medicine, National Cheng-Kung University Medical Center, Tainan, Taiwan,
†Institute of Clinical Medicine, College of Medicine, National Cheng-Kung University, Tainan, Taiwan, and
‡Department of Emergency Medicine, Department of Internal Medicine, National Cheng-Kung University Medical Center, Tainan, Taiwan

ABSTRACT

The aim of the present study was to assess the association between invasive PWV (pulse wave velocity), serum levels of MMPs (matrix metalloproteinases) and the echocardiographic severity and calcification score of degenerative AS (aortic stenosis). We enrolled 30 patients (16 males; age, 61.3 ± 8.2 years) diagnosed with degenerative AS and an additional 30 age- and sex-matched control patients. Invasive PWV methods with a pigtail catheter and double-channel recording were performed in both groups in our catheterization laboratory. We scored the severity of calcification at the AV (aortic valve) during two-dimensional echocardiography. The association between the trans-valvular pressure gradient, the severity of calcification of the AV and the value of PWV were analysed. We also analysed the serum levels of MMP-9, MMP-3 and TIMP-1 (tissue inhibitor of metalloproteinases-1) in these patients. In the group with degenerative AS, mean AV pressure gradients (56.0 compared with 9.5 mmHg; \( P < 0.001 \)) and calcified AV scoring (3.3 ± 1.2 compared with 2.1 ± 0.9; \( P < 0.001 \)) were higher than in the control group. In addition, PWV was faster in the group with degenerative AS (15.5 ± 3.8 compared with 8.0 ± 2.7 m/s; \( P = 0.001 \)). After being adjusted for age, sex, mean blood pressure and left ventricular function, both the AV pressure gradient and the severity of calcification were strongly correlated with PWV (\( R = 0.706, P < 0.0001 \), and \( R = 0.561, P = 0.03 \) respectively). In addition, the serum levels of MMP-9, MMP-3 and TIMP-1 were all significantly higher and correlated with PWV in the group with AS (all \( P < 0.05 \)). With higher serum levels of MMPs and their inhibitors, we found that this invasive measurement of PWV was associated strongly with the pressure gradient and calcification of AV. More advanced degenerative changes in AV was probably associated with more severe aortic arteriosclerosis.

Key words: aortic stenosis, calcification, echocardiography, matrix metalloprotei

Abbreviations: AS, aortic stenosis; AV, aortic valve; BMI, body mass index; BP, blood pressure; CAD, coronary artery disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LV, left ventricular; MMP, matrix metalloprotei

Correspondence: Dr Jyh-Hong Chen (email jyhhong@mail.ncku.edu.tw).
INTRODUCTION

The prevalence of degenerative AS (aortic stenosis) is increasing among aging patients [1,2]. With a more advanced degenerative process, the opening of the orifice will be limited and the pressure gradient between the AVs (aortic valves) will increase. Higher trans-valvular pressure gradient across the AV can cause angina pectoris, congestive heart failure, syncope and even sudden cardiac death [2]. Trans-valvular Doppler echocardiography [3] and invasive catheterization pressure gradient measurement [4] are the methods most commonly used in AS evaluation; however, the measurements are sometimes difficult or unavailable, especially in patients with poor echocardiographic study windows or severely calcified AVs.

Increased collagen and calcium deposits were found in specimens of degenerative AVs [5]. The same process also occurs in large vessels, with increased stiffness and decreased compliance during aging [5,6]. The degree of increased vessel stiffness can be measured by PWV (pulse wave velocity) [7,8]. The aortic PWV between the carotid and femoral arteries is commonly used to evaluate arterial stiffness, and can imply the compliance and elasticity of vascular structures. Several population-based studies have reported that this parameter could predict the presence of cardiovascular events and mortality in hypertensive patients [9,10], end-stage renal disease patients [11] and elderly subjects [12]. The severity of calcification at the AV evaluated from the two-dimensional echocardiography has been found to be a useful predictor for rapid degeneration of AV stenosis [2]. However, the associations between the invasive catheter-based measurements of PWV, the severity of AV calcification and the severity of AS are still not clear.

MMPs (matrix metalloproteinases), such as interstitial collagenase, gelatinase and stromelysin-1, can degrade the extracellular matrix and are identified extensively in human atherosclerotic lesions [13,14]. Stromelysin-1, also called MMP-3, was found to be able to cleave many of the extracellular matrix components and to enhance the activity of other MMPs [15]. This family of enzymes and their inhibitors play a pivotal role in the physiological and pathological events associated with connective tissue remodelling, including changes in arterial wall composition and the degree of great artery stiffening [16]. Compared with normal AVs, there is an extensive cellular infiltrate consisting of macrophages, lymphocytes and fibroblasts in the degenerative AVs obtained during AV replacement [17]. Two studies using immunohistochemical staining [17,18] have shown increased expressions of MMP-1, -2, -3 and -9 in degenerative AVs.

We hypothesized that patients with degenerative AS would have a similar atherosclerotic process in the aorta. More advanced degenerative changes in the AV might also be associated with more severe aortic arteriosclerosis. Thus increased PWV may occur in those with more advanced AS and with higher trans-valvular AV pressure gradients. We also hypothesized that those with advanced AS would have increased serum MMP and TIMP (tissue inhibitor of metalloproteinases) levels as the arteriosclerosis process progressed and these enzymes might also be associated with increased PWV.

In the present study, we evaluated the association of the PWV measured by an invasive method and the severity of AV calcification and the degree of trans-valvular pressure gradient by using two-dimensional Doppler echocardiography in AS patients. The association between PWV and serum levels of MMP and TIMP has also been evaluated.

This work was presented in part at EUROECHO 7, held at Barcelona, Spain, in December 2003, and at the 74th European Atherosclerosis Society, held at Seville, Spain, in April 2004, and subsequently published in abstract form [18a,18b].

METHODS

Patient selection

Thirty-three patients (17 males; age, 63.3 $\pm$ 8.7 years) diagnosed with degenerative AS, who had undergone echocardiography and catheterization at our Medical Center, were enrolled. We also selected 30 patients, who received diagnostic catheterization for other reasons. Advanced hypertensive patients (stage II of World Health Organization classification), who had severe complications, patients with secondary hypertension, impaired renal functions with serum creatinine greater than 2.0 mg/dl, those subjects with arrhythmia, including atrial fibrillation, or cerebrovascular disease by routine physical and laboratory examinations, were all excluded. Because aortic regurgitation could modify the transmission of the pulse wave, we also excluded patients who had more than mild degree of severity of concomitant aortic regurgitation. Among these 63 patients, 15 (23.8 %) patients were treated with antihypertensive drugs (calcium antagonist for nine patients and $\beta$-blockade for the other six patients; all were single agents).

The protocol of the present study was approved by the Hospital Ethics Committee, and written informed consent was obtained from all the patients.

PWV

An invasive method for PWV measurement was used in the present study. A 7-French puncture sheath was inserted into the right femoral artery and a 6-French pigtail catheter was used for pressure wave recording. Under fluoroscopy, the tip of the pigtail catheter was placed at the level of the descending aorta just below the opening of the left subclavian artery. Double-channel pressure wave recordings were obtained simultaneously.
from the pigtail and the side arm of the femoral sheath. Time from the R-wave of the ECG to the foot of the pressure wave upstroke was established. The average of five wave measurements was used for the calculation. The transition time between two waves was derived from time difference between the two waves. The distance between the sampling sites was measured by the length of the pigtail catheter from the tip to the insertion site in the femoral sheath. PWV was calculated from distance divided by transition time (Figure 1).

Echocardiography

Echocardiography was performed before the catheterization procedures with a 2.5 MHz transducer connected to an ultrasound system (Hewlett-Packard Sonos 1000; Andover, MA, U.S.A.). The echocardiographic images were reviewed off-line for the determination of M-mode dimensions in accordance with American Society of Echocardiography standards [19]. LV (left ventricular) mass index was calculated using the M-mode-derived formula described by Woythaler et al. [20]. Standard images of the left ventricle were reviewed to identify wall motion abnormalities. LV ejection fraction was estimated by the M-mode image. Doppler echocardiographic evaluation of AS followed the standard methods [21]. The peak velocity beneath the aortic valve ($V_1$) was assessed by pulsed Doppler from the apical five-chamber view, with the sample volume beneath the aortic annulus just proximal to the region of flow acceleration. The peak velocity across the valve ($V_2$) was recorded from multiple windows using continuous wave Doppler. Peak velocities were recorded as the average of three to five measurements. Pressure gradients were calculated using the modified Bernoulli equation:

\[
\text{Gradient (mmHg)} = 4 \times \left( V_2^2 - V_1^2 \right).
\]

AV leaflet morphology was assessed qualitatively during the echocardiographic studies by one observer blinded to the severity of AS. AV leaflets were evaluated from the parasternal long- and short-axis views. Calcification of the aortic leaflets was described as follows: 1, none; 2, \( \geq 1 \) localized area of increased reflectivity, but no areas of dense ‘calcification’; 3, markedly increased reflectivity (calcification) in one leaflet, but \( \leq 2 \) grade 2 changes in the other leaflets; 4, markedly increased reflectivity in two leaflets, but \( \leq 2 \) grade 2 changes in the third leaflet; 5, moderately increased reflectivity in all leaflets; 6, severely increased reflectivity in all leaflets [2].

Clinical data

Medical records were reviewed for clinical variables. CAD (coronary artery disease) was considered present if selective coronary angiography showed significant stenosis (> 50%). Hypertension was considered to be present if a patient was receiving antihypertensive therapy or had two or more BP (blood pressure) recordings of 140/90 mmHg. Arterial BP was recorded routinely while evaluating PWV in the catheterization laboratory; this
value was used to evaluate whether BP was associated with the value of PWV.

Measurements of MMP-9, MMP-3 and TIMP-1
Serum levels of MMP-9, MMP-3 and TIMP-1 were measured in peripheral blood samples using a commercially available one-step sandwich enzyme immunoassay with monoclonal antibodies against each substance [22–25], according to the manufacturer’s instructions (CN Biosciences, San Diego, CA, U.S.A.). The enzyme immunoassay system for MMP-9 and MMP-3 is capable of measuring precursor and active forms of MMPs, as well as the forms of MMPs complexed with TIMP; however, MMPs exist only as the precursor forms in human blood. This system recognizes with high immunoreactivity (approx. 50 %) free active MMPs [23,24]. The enzyme immunoassay system for TIMP-1 can detect free TIMP-1 and TIMP-1 complexed with active forms of MMPs [24].

Statistical analysis
Values are expressed as means ± S.D. if normally distributed, or median (range) if not normally distributed. For normally distributed variables, the frequencies between normally distributed variables, the frequencies between groups were compared by χ² analysis. One-way ANOVA, followed by Fisher’s protected least-significant difference test, was used for comparison of mean values of continuous variables (expressed as means ± S.D.) among groups. For non-parametric analysis, the Mann-Whitney U-test was used to evaluate the difference in levels between AS and control groups. ROC (receiver operator characteristic) analysis was performed to determine the best cut-off value of PWV to detect significant AS with trans-valvular pressure gradient ≥ 50 mmHg. Because the values of trans-valvular pressure gradients were not normally distributed, the associations between the pressure gradient values, severity of the calcification at the AVs and the PWVs were analysed by the non-parametric Spearman’s rank correlation test. The predictive value for significant AS was assessed by Cox’s proportional hazard analysis with the following factors as categorical variants: multiple (> double-vessel CAD) coronary arteries with stenosis, previous myocardial infarction, lower LV ejection fraction, lower LV hypertrophy, including thickened interventricular septum diameter (> 12.6 mm) and higher LV mass index (> 124 g/m²). The multivariate Cox analysis using the forward stepwise method included only the covariates that predicted significant AS and their P values were < 0.2 in the forward stepwise selection univariate analysis. Statistical significance was defined as P < 0.05. All analyses were performed with a software program (SPSS 8.1 for Windows; SPSS, Chicago, IL, U.S.A.).

RESULTS
Baseline data of patients with or without AS are shown in Table 1. Three patients with AS were excluded due to atrial fibrillation during the invasive study (two patients) or renal function impairment after catheterization study (one patient). Finally, 30 patients were enrolled in the AS group and an additional 30 patients in the control group. There was no significant difference in the average of age and sex distribution between the two groups. The frequency of CAD, hypertension, diabetes mellitus, hypercholesterolaemia and histories of smoking behaviour were similar in both groups. Patients in these two groups also had similar systolic and diastolic BP, fasting sugar, BMI and lipid profile, including total cholesterol, HDL and LDL levels. In addition, the distribution of the number of diseased coronary arteries was very similar in both groups.

The two-dimensional echocardiography variables are shown in Table 2. The mean values for the thickness of
Table 2 Echocardiographic characteristics and PWVs of patients without or with AS
Values are as means ± S.D., or median (range). IVSD, interventricular septum diameter; LVEF, LV ejection fraction.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients with AS (n = 30)</th>
<th>Patients without AS (n = 30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two-dimensional echocardiogram</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVSD (mm)</td>
<td>14 ± 4</td>
<td>12 ± 5</td>
<td>0.08</td>
</tr>
<tr>
<td>LV mass index (g/m²)</td>
<td>155.3 ± 69.5</td>
<td>132.1 ± 40.5</td>
<td>0.05</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>50.5 ± 11.3</td>
<td>61.6 ± 12.1</td>
<td>0.07</td>
</tr>
<tr>
<td>AV pressure gradient (mmHg)</td>
<td>56.0 (27–105)</td>
<td>9.5 (5.5–16)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PWV (m/s)</td>
<td>15.5 ± 3.8</td>
<td>8.0 ± 2.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AV calcification (grade)</td>
<td>3.3 ± 1.2</td>
<td>2.1 ± 0.9</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Figure 2 ROC curves using PWV in the detection of severe AS
Area under curve, 0.73 ± 0.12, P = 0.02.

interventricular septum and LV mass index were not significantly higher in the degenerative AS group compared with the controls. The mean value of LV ejection fraction via M-mode evaluation was similar in both groups. The patients with degenerative AS, as expected, had higher mean AV pressure gradients when compared with the control group. The calcified AV scoring was also significantly higher in the degenerative AS group compared with control group.

The values of invasively measured PWV from the catheterization study in the degenerative AS group and control group ranged from 8.2–24.3 m/s and 4.6–12.3 m/s respectively, and PWV was faster in the degenerative AS group compared with the control group (Table 2).

By performing ROC analysis, the best cut-off value of PWV was chosen as 12.3 m/s to detect a significant AS with a trans-valvular pressure gradient ≥ 50 mmHg) respectively. In a univariate analysis, we found that increased PWV, diabetes mellitus, systemic hypertension, more severe coronary disease and increased AV calcification score in the echocardiographic study were all significant predictors for severe AS. After multivariate analysis, we found that only increased PWV, diabetes mellitus and systemic hypertension remained independent predictors for severe AS (Table 3). After adjusting for age, sex, severity of CAD, traditional risk factors and LV functions, the AV pressure gradient had a strong positive association with PWV in the degenerative AS group (R = 0.706, P < 0.0001; Figure 3a). In addition, the scoring of the severity of AS calcification was also positively correlated with the PWV (R = 0.561, P = 0.03; Figure 3b).

In addition, we compared the serum MMP-9, MMP-3 and TIMP-1 levels among these two groups (Figure 4). The levels of MMP-9 (91.4 ± 20.4 and 68.3 ± 16.9 ng/dl respectively) and MMP-3 (32.6 ± 4.4 and 25.3 ± 5.2 ng/dl respectively) in the peripheral blood samples were both greater in the AS group than in the control group. The levels of TIMP-1 in the peripheral blood samples (87.6 ± 9.4 and 94.0 ± 11.2 ng/dl respectively) were also greater in the AS group. The level of TIMP-1 was correlated with MMP-9 and MMP-3 levels among the AS group (R = 0.42 and 0.48, respectively; both p < 0.01). Finally, the levels of MMP-9, MMP-3 and TIMP-1 were all positively and significantly correlated with the PWV in the AS group (R = 0.52, 0.46 and 0.48 respectively; P = 0.02, 0.03 and 0.02 respectively).

**DISCUSSION**

In this case-controlled study, we have analysed the association of AV pressure gradient with PWV in patients with degenerative AS. After being adjusted for age, sex, traditional risk factors and LV functions, we found...
that a strong correlation existed. In addition, we also demonstrated the association of increased PWV and higher scoring of calcification at the AV observed from the two-dimensional echocardiography. Higher levels of MMP-9, MMP-3 and TIMP-1 in the peripheral blood samples and good correlations between these levels and PWV were observed in patients in the AS group. These results indicated that, during the degenerative process, calcium deposition played an important remodelling role not only in the AV, but also in the aorta. The degenerative process of the AV was correlated strongly with the atherosclerotic process occurring in the aorta.

Our present study demonstrates a strong association between the higher calcium scoring observed by echocardiography and the increased PWV measured using an invasive catheterization method. Increased calcium and collagen deposition have been detected extensively in the degenerative AV and the atherosclerotic aorta [1]. Dysfunction of endothelial cells was observed in patients with advanced calcification and atherosclerosis [26]. Arterial stiffness measured by PWV has been used widely to evaluate arterial aging and to predict the presence of cardiovascular events, especially in elderly subjects [12]. Among patients with end-stage renal disease, arterial stiffness and the severity of arterial calcification have been demonstrated as important diagnostic risk factors with respects to cardiovascular mortality or total mortality [11]. For degenerative and calcified AVs, an increased calcium score, a limited excursion score and a higher severity score all indicated an accelerated progression rate of aortic opening narrowing and an increased mortality rate [2]. Some evidence has shown that pathological features of AS resemble atherosclerosis with calcification [26, 27]. This could partially explain the strong association of increased severity in AS and the higher calcification score measured by echocardiography with PWV in the present study.

Several confounding factors has been reported to have an impact on arterial stiffness, including age, sex, mean BP, antihypertensive medication, smoking and diabetes mellitus history [28–32]. In our analysis model, we used

\[ \text{Correlation between PWV, after adjustment for age, sex, traditional risk factors and LV function, and (a) the AV pressure gradient and (b) the scoring of AV calcification} \]
univariate and multivariate Cox analysis to define the possible confounding effects by age, sex, traditional risk factor and LV function. We found that increased PWV, diabetes mellitus and systemic hypertension were the most important and independent risk factors for severe AS. Our present results indicated that, with more advanced degenerative changes in the AV, it would probably be associated with more severe aortic atherosclerosis. However, an immediate relief on LV diastolic chamber stiffness has been found after AV replacement for AS [33]. As a result, we could not completely exclude the effect of ventricular stiffness due to severe AS, which might contribute in the velocity of pulse transmission in large arteries.

A recent study has investigated the genetic mutation of MMP-3 and its contribution to the age-related large artery stiffening [16]. These workers used a non-invasive large artery stiffness method and found that patients carrying an MMP-3 genotype with the 5A allele, which might carry increased MMP-3 activity, had increased levels of age-related arterial stiffening and matrix accumulation. Our present study population also demonstrated that patients with AS had higher levels of MMP-3 in the peripheral circulation. These results favour a persistent MMP activation state within the calcified valve which probably contributes to the valvular remodelling process in the setting of developing AS, and support the similar pathophysiological changes between AV degeneration and large artery stiffening.

Limitations of the study

Similar to previous studies of AS, the present study was limited by the small number of patients. In addition, we did not have any tissue biopsies from the myocardium, AV or the aorta, which might be helpful in interpreting the relationship between AS and other confounding factors, such as changes in structure and function of the left ventricle. Furthermore, we compared these patients with a control group well-matched for risk factors. This might imply that the results are only applicable to a limited group of elderly patients, which was of higher prevalence among the AS population.

Conclusion

In this invasive study, we analysed the association of the AV pressure gradient and the calcified score with PWV in a relatively aged AS population and found that a strong correlation existed between them. More advanced degenerative changes in the AV was probably associated with more severe aortic atherosclerosis.

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