Original article

Systolic Volume Index by Doppler Echocardiography Is an Useful Marker for Stratification and Prognostic Evaluation in Patients With Severe Aortic Stenosis and Preserved Ejection Fraction

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A B S T R A C T

Introduction and objectives: The prognosis of patients with severe aortic stenosis, low aortic gradient and preserved ejection fraction is controversial. Our study analyzed the prognosis of these patients and its relation to pressure gradient and aortic valve flow.

Methods: We performed a retrospective cohort study of 363 consecutive patients with severe aortic stenosis and preserved ejection fraction, divided into 4 groups, based on the presence of a systolic volume index greater or lower than 35 ml/m² and the presence of a mean aortic gradient greater or lower than 40 mmHg. Group I: normal flow, high gradient (n=169, 47%); group II: normal flow, low gradient (n=98, 27%); group III: low flow, high gradient (n=54, 15%), and group IV: low flow, low gradient (n=42, 12%). The primary endpoint was overall mortality.

Results: Independent risk factors for mortality were age (hazard ratio = 1.04; 95% confidence interval, 1.01-1.08) and atrial fibrillation (hazard ratio = 2.21; 95% confidence interval, 1.24-3.94). Surgical treatment was associated with longer survival in all groups (hazard ratio = 0.25; 95% confidence interval: 0.13-0.49). Mortality was higher in patients with low flow than in those with normal flow (26.6% vs 13.6%; P < 0.004). The most favorable mean prognosis was found in group II (hazard ratio = 0.4; 95% confidence interval, 0.2-0.9).

Conclusions: Patients with severe aortic stenosis, normal ejection fraction and low aortic flow have a worse prognosis. Analysis of aortic flow by Doppler echocardiography is useful in risk stratification and therapeutic decision-making in patients with aortic stenosis.

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El análisis del flujo aórtico por ecocardiografía Doppler es útil en la estratificación pronóstica de los pacientes con estenosis aórtica grave con fracción de eyeción normal

R E S U M E N

Introducción y objetivos: El pronóstico de los pacientes con estenosis aórtica grave con bajo gradiente aórtico y fracción de eyeción normal es controvertido. Nuestro estudio analiza el pronóstico de estos pacientes y su relación con el gradiente de presión y el flujo valvular aórtico.

Métodos: Cohorte retrospectiva de 363 pacientes consecutivos con estenosis aórtica grave y fracción de eyeción normal, dividida en cuatro grupos según índice de volumen sistólico mayor o menor que 35 ml/m² y gradiente aórtico medio mayor o menor que 40 mmHg. Grupo I, flujo normal y gradiente elevado (n = 169; 47%); grupo II, flujo normal y bajo gradiente (n = 98; 27%); grupo III, bajo flujo y gradiente elevado (n = 54; 15%); y grupo IV, bajo flujo y bajo gradiente (n = 42; 12%). El objetivo primario es la mortalidad total.

Resultados: Los factores de riesgo independientes de mortalidad son la edad (hazard ratio = 1.04; intervalo de confianza del 95%, 1.01-1.08) y la fibrilación auricular (hazard ratio = 2.21; intervalo de confianza del 95%, 1.24-3.94). El tratamiento quirúrgico se asocia a mayor supervivencia en todos los grupos (hazard ratio = 0,25; intervalo de confianza del 95%, 0,13-0,49). Los pacientes con bajo flujo presentan mayor mortalidad que los pacientes con flujo normal (el 26,6 frente al 13,6%; p = 0,004). El grupo II muestra mejor pronóstico (hazard ratio = 0,4; intervalo de confianza del 95%, 0,2-0,9).

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Conclusions: Los pacientes con estenosis aórtica grave, fracción de eyeción normal y bajo flujo aórtico presentan peor pronóstico. El análisis del flujo aórtico mediante ecocardiografía Doppler es útil en la estratificación de riesgo y en la toma de decisiones terapéuticas en pacientes con estenosis aórtica.

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tests or Fisher's exact tests for categorical variables. The primary end-point of this study was overall mortality. Survival was assessed using Kaplan-Meier curves for each group. Differences among groups in terms of risk factors were determined using log rank tests. The effects of clinical and echocardiographic variables on survival were determined using Cox proportional hazards models, including aortic valve replacement as a time-dependent variable. All possible associated variables and those that resulted in a P-value < .2 in the univariate analysis were included in the multivariate analysis. We used SPSS statistical software for Windows®, version 15.0, for all statistical analyses.

RESULTS

Our study population included a total of 363 consecutive patients: 186 women and 177 men; median age, 77 [71–82] years. Patients were monitored for a median of 25.3 [14.8–33.2] months after inclusion in the study. Follow-up was not possible in 4 of the 363 patients (1.1%).

Table 1 shows the baseline characteristics of the study population. Patients with a low gradient (groups II and IV) had a greater frequency of coronary artery disease, with the greatest difference observed between groups I and IV. There were no significant differences between groups in terms of age, sex, body mass index, body surface area, hypertension, tobacco use, or hypercholesterolemia. Patients with severe AS and low flow (groups III and IV) were older (78 years vs 76 years; P = .047), had a higher prevalence of diabetes and AF, and the proportion of women was higher (57.3% vs 42.7%; P = .167).

Table 2 displays the results of severity of AS, arterial afterload, and global afterload. By definition, groups I and III had higher gradients. Patients with a high gradient and low flow (group III) had lower AVA values, a lower AVA index, and a lower integral ratio, indicating more severe stenosis than in the remaining groups. Patients in group IV (low gradient and low flow) had more severe stenosis than those in group II, who had a low gradient but normal flow, despite the lower gradients observed in this group.

Patients with normal flow and low gradient (group II) had less severe stenosis than the remaining groups. In this group, mean
systolic pressure was higher than in the remaining groups. Patients with low flow (groups III and IV) had higher values of systemic vascular resistance and lower values of arterial compliance, suggesting a greater vascular afterload. Global afterload in the LV (valvuloaortic impedance) was also greater in low flow patients, especially in those with a high gradient (group III).

Table 3 compares LV geometry and systolic function parameters among the 4 groups. Patients with low flow (groups III and IV) had smaller ventricular cavities than those with normal flow (groups I and II) (left ventricular telediastolic diameter: 46 mm vs 48 mm; P=0.012; left ventricular telediastolic index: 57 mL vs 63 mL; P=0.023). There were no differences among the distinct groups in terms of hypertrophy, and all showed concentric remodelling, which was more pronounced in patients with a high gradient and low flow (group III).

We observed no differences among groups in terms of EF, although midwall fractional shortening was lower in patients with low flow and high gradient (group III) and higher in patients with normal flow and low gradient (group II). Patients with low flow (groups III and IV) also had lower values for stroke work, cardiac output, and cardiac index, with a compensatory increase in heart rate. Globally, SVI was lower in patients with AF (38.0 mL/m² [95%CI, 35.2-40.9 mL/m²] vs 43.0 mL/m² [95%CI, 41.9-44.0 mL/m²]). When analysis was performed by patient group, only group IV was not significantly different when patients with and without AF were compared (group I: 43.8 mL/m² [95%CI, 37.8-51.1 mL/m²]; group II: 47.0 mL/m² [95%CI, 41.0-52.8 mL/m²]; group III: 37.0 mL/m² [95%CI, 36.0-44.4 mL/m²] vs 44.0 mL/m² [95%CI, 38.7-48.2 mL/m²]; group III: 28.1 mL/m² [95%CI, 27.0-32.0 mL/m²] vs 32.0 mL/m² [95%CI, 29.0-34.0 mL/m²]; group IV: 31.0 mL/m² [95%CI, 26.1-33.5 mL/m²] vs 32.0 mL/m² [95%CI, 29.2-34.0 mL/m²]).

Table 4 summarizes the clinical follow-up for all 4 groups. A total of 216 patients (60.2%) underwent aortic valve replacement.

### Table 3: Echocardiographic Results of the Geometry and Systolic Function of the Left Ventricle

<table>
<thead>
<tr>
<th></th>
<th>Group I (normal flow, high gradient)</th>
<th>Group II (normal flow, low gradient)</th>
<th>Group III (low flow, high gradient)</th>
<th>Group IV (low flow, low gradient)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVOTD diameter, mm</td>
<td>20 (20-21)</td>
<td>20.3 (20-21)</td>
<td>19 (19-20)</td>
<td>20 (19-20)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>LVTDD, mm</td>
<td>48 (47-49)</td>
<td>49 (48-50)</td>
<td>46 (44-48)</td>
<td>46 (44-49)</td>
<td>.067</td>
</tr>
<tr>
<td>LVTSD, mm</td>
<td>30 (30-31)</td>
<td>31 (30-32)</td>
<td>30 (28-31)</td>
<td>30 (28-32)</td>
<td>.253</td>
</tr>
<tr>
<td>SV, mL/beat</td>
<td>84 (82-87)</td>
<td>77 (75-80)</td>
<td>54 (52-57)</td>
<td>54 (52-57)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>SV index, mL/beat/m²</td>
<td>48 (46-49)</td>
<td>44 (43-45)</td>
<td>31 (30-32)</td>
<td>31 (30-32)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>LVTDD index, mL/m²</td>
<td>62 (59-65)</td>
<td>65 (61-68)</td>
<td>57 (51-63)</td>
<td>58 (52-65)</td>
<td>.093</td>
</tr>
<tr>
<td>Posterior wall, mm</td>
<td>12.6 (12.3-12.9)</td>
<td>11.9 (11.3-12.3)</td>
<td>13.3 (12.7-13.9)</td>
<td>12.5 (11.7-13.2)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Septum, mm</td>
<td>143 (140-146)</td>
<td>13.2 (12.9-13.6)</td>
<td>14.4 (13.8-14.9)</td>
<td>13.5 (12.9-14.1)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Adjusted LV mass, g/m²</td>
<td>150 (144-157)</td>
<td>142 (134-149)</td>
<td>148 (136-159)</td>
<td>139 (127-152)</td>
<td>.228</td>
</tr>
<tr>
<td>Relative parietal thickness</td>
<td>0.53 (0.51-0.55)</td>
<td>0.49 (0.5-0.5)</td>
<td>0.59 (0.54-0.7)</td>
<td>0.55 (0.51-0.6)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>66 (64-67)</td>
<td>64 (62-66)</td>
<td>65 (63-67)</td>
<td>64 (62-67)</td>
<td>.418</td>
</tr>
<tr>
<td>SW, %</td>
<td>14.9 (14.5-15.3)</td>
<td>15.5 (15.0-16.0)</td>
<td>13.8 (13.1-14.5)</td>
<td>14.6 (13.8-15.4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>SW, g/m²</td>
<td>175 (169-181)</td>
<td>137 (131-142)</td>
<td>113 (107-119)</td>
<td>91 (84-98)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>68 (66-70)</td>
<td>68 (65-70)</td>
<td>78 (73-82)</td>
<td>80 (74-87)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CO, L/min</td>
<td>5 (5.5-5.7)</td>
<td>5.2 (5.0-5.4)</td>
<td>4.2 (3.9-4.4)</td>
<td>4.3 (4.0-4.7)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cl, L/min/m²</td>
<td>3 (3.1-3.3)</td>
<td>3.0 (2.8-3.1)</td>
<td>2.4 (2.2-2.5)</td>
<td>2.5 (2.3-2.7)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

CI, cardiac index; CO, cardiac output; HR, heart rate; LV, left ventricular; LVOT, left ventricular outflow tract; LVTDD, left ventricular telediastolic diameter; LVTSD, left ventricular telesystolic diameter; MFS, midwall fractional shortening; SV, systolic volume; SW, stroke work.

Values are expressed as means (95% confidence interval).

### Table 4: Clinical Results

<table>
<thead>
<tr>
<th></th>
<th>Group I (normal flow, high gradient)</th>
<th>Group II (normal flow, low gradient)</th>
<th>Group III (low flow, high gradient)</th>
<th>Group IV (low flow, low gradient)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVR</td>
<td>112 (66.3) [59.1-73.4]</td>
<td>51 (53.1) [43.1-63.1]</td>
<td>34 (65.4) [52.7-78.3]</td>
<td>19 (45.2) [30.2-60.3]</td>
<td></td>
</tr>
<tr>
<td>Previous bypass or stent</td>
<td>3 (2.7)</td>
<td>6 (11.8)</td>
<td>0 (0)</td>
<td>2 (10.5)</td>
<td></td>
</tr>
<tr>
<td>Combined surgery</td>
<td>33 (29.5) [21.0-37.9]</td>
<td>24 (47.1) [33.4-60.8]</td>
<td>14 (41.2) [24.6-57.7]</td>
<td>9 (47.4) [24.9-69.8]</td>
<td>.667</td>
</tr>
<tr>
<td>Non-AVR deaths</td>
<td>21 (36.8) [24.3-49.4]</td>
<td>8 (17.8) [6.6-29.0]</td>
<td>9 (50.0) [26.9-73.1]</td>
<td>10 (43.5) [23.2-65.5]</td>
<td>.038</td>
</tr>
<tr>
<td>AVR deaths</td>
<td>6 (5.4) [1.2-9.5]</td>
<td>1 (2.0) [0.1-10.5]</td>
<td>3 (8.8) [1.9-23.7]</td>
<td>3 (15.8) [3.4-39.6]</td>
<td>.126</td>
</tr>
<tr>
<td>Perioperative mortality</td>
<td>3 (2.8) [0.6-7.6]</td>
<td>0 (0.0) [0.0-7.0]</td>
<td>2 (5.9) [0.7-19.7]</td>
<td>1 (5.6) [0.1-26.0]</td>
<td>.217</td>
</tr>
<tr>
<td>Total mortality</td>
<td>27 (16.0) [10.5-21.5]</td>
<td>9 (5.4) [4.4-17.1]</td>
<td>12 (23.1) [12.5-36.8]</td>
<td>13 (31.0) [17.0-44.9]</td>
<td>.011</td>
</tr>
</tbody>
</table>

AVR, aortic valve replacement.

Values are expressed as (%) (95% confidence interval) or median [interquartile range].
with a mean postoperative follow-up of 20.9 [11.2–29.6] months. The median follow-up in patients receiving medical treatment was 20.1 [9.2–30.9] months. Patients who underwent surgery were significantly younger in all groups. Patients with a low gradient (groups II and IV) underwent fewer surgical interventions than did patients with a high gradient (50.7% vs 65.8%; P<0.005). During follow-up, 61 patients died (16.8%). The mortality rate in patients who underwent surgery was 6.0% (13 out of 216) as compared to 33.6% (48 out of 143) of those treated using a conservative approach. Patients with low flow (groups III and IV) had greater mortality rates than did patients with normal flow (total mortality: 26.6% vs 13.6%; P=0.004). In patients who underwent surgery, mortality was lower in all 4 groups than in those who received conservative treatment (Figure). Patients in group II had the lowest mortality rate during the follow-up period, and this difference was even larger in patients who underwent surgery. Six of the surgical patients died during the postoperative period.

Table 5 shows the predictors for overall mortality in the univariate and multivariate analyses. The factors associated with an increase in mortality in the univariate analysis were age, medical treatment, SVI, stroke work, and AF, but not mean gradient or valvular aortic valve impedance.

In the multivariate analysis, the independent risk factors found were age and AF, whereas surgical treatment (aortic valve replacement with or without revascularization) significantly reduced mortality (HR=0.25; 95%CI, 0.13-0.49). Sex, SVI, and mean aortic gradient failed to reach statistical significance, although the results tended to indicate that a higher mortality rate was associated with male sex, decreased SVI, and increased gradient. Stroke work, valvular ventricular impedance, and AVA were closely correlated with SVI (r=0.9) and were not included in the model 2.

In model 2, differences were assessed between the 4 different study groups. Group II (low gradient, normal flow) had the lowest observed mortality rate (HR=0.4; 95%CI, 0.2-0.9; P=0.02). We found no interaction between the variables of patient group and surgical intervention.

Hypertension, diabetes mellitus, coronary arterial disease, concomitant coronary revascularization surgery, systemic arterial compliance, systemic vascular resistance, EF, midwall fractional shortening, relative wall thickness, and cardiac output were not significantly related to mortality.

**DISCUSSION**

The most relevant findings of our study are that in patients with severe AS (defined as an AVA<1 cm²) and normal EF, decreased aortic valve flow is an indicator of a poorer prognosis, and that surgical treatment reduces mortality rates in all study groups (Figure).
Low Flow Aortic Stenosis

Our study showed that 27% of patients had low flow AS, which is in agreement with previous publications.4,6,13 In our sample, 56% of patients with low flow had a high gradient (group III), which confirms that low flow is not synonymous with low aortic gradient. Our results reaffirm the previous hypothesis4–10 that these patients with severe AS and low flow have a greater afterload, resulting from the combination of valvular obstruction and increased peripheral resistance, which would produce increased concentric ventricular remodelling and myocardial dysfunction.

As in past studies,14 we observed 2 clearly differentiated groups of low aortic flow; one with less severe cases of stenosis and low gradients (group IV) and another with a greater severity of valvular obstruction and high gradients (group III). In both groups, we observed a decrease in SV and a worse prognosis than in other patients with severe AS and normal flow. In this context, both low-flow groups had a higher prevalence of AF than did patients with normal flow rates. AF may reflect more advanced disease, given its predictive value in the multivariate analysis, in addition to the fact that the loss in atrial contraction can reduce ventricular filling and contribute to reducing cardiac output. Globally, patients with AF had a lower SVI (38 vs 43). In a previous study, AF was associated with increased survival following surgery in patients with severe AS and ventricular dysfunction.17 In a more recent study,18 asymptomatic patients with severe AS and normal EF (and exclusion of patients with AF), those with low flow had a significantly worse prognosis during follow-up. In our study, AF acted as an independent risk factor for mortality.

Group IV patients (low flow and low gradient) who underwent surgery had a lower survival rate than group III patients undergoing surgery (low flow and high gradient). Group III patients had critical AS, and the decrease in contractile function and lower SV may be correlated with the severity of valvular obstruction, whereas the results in group IV (with less severe stenosis) suggest the coexistence of other factors in addition to valvular obstruction. In our study population, and similar to the study by Clavel et al.,19 coronary arterial disease was significantly more prevalent in this group. Group IV constituted a more heterogeneous group with greater comorbidity (age, diabetes, ischemic heart disease, AF...), with a tendency for a higher overall mortality rate, fewer indications for surgery, and inferior surgery results compared with the remaining groups. On a possibly related topic, Hermann et al.20 described a greater degree of myocardial fibrosis in the subendocardium and decreased longitudinal function (calculated using 2-dimensional strain) in patients with low flow and a low gradient, anomalies which were irreversible following the aortic valve replacement, and which were considered to be the cause of the worse postoperative results in these patients. Recently, in a prospective study, Adda et al.21 also observed a severe deterioration in longitudinal function of the left ventricle using 2-dimensional strain in patients with a low gradient and low flow.

Severe Low-Gradient Aortic Stenosis

In our study, 39% of patients had severe low gradient AS. Most of our patients with low gradients had normal aortic flow (70%; group II). These patients had a less severe degree of stenosis and reduced ventricular remodelling with better indexes of heart function, and consequently a better prognosis, than other patients from the remaining groups. In patients with severe AS and low gradient, 30% had low flow (group IV, 12% of all patients with AS) and had a worse prognosis than those with a low gradient and normal flow (group II) and had a similar prognosis to patients with high gradients. These findings are in agreement with those of Dumesnil et al.14 and differ from those of a study by Jander et al.22 in which patients with severe AS, low gradient, and normal or low aortic flow had a

### Table 5

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>361</td>
<td>96</td>
<td>52</td>
<td>42</td>
</tr>
<tr>
<td>Age, years</td>
<td>.93</td>
<td>.010</td>
<td>.027</td>
<td>.002</td>
</tr>
<tr>
<td>Surgical treatment</td>
<td>.93</td>
<td>.010</td>
<td>.027</td>
<td>.002</td>
</tr>
<tr>
<td>Stroke work</td>
<td>.015</td>
<td>.019</td>
<td>.206</td>
<td>.042</td>
</tr>
<tr>
<td>Systolic volume index</td>
<td>0.96</td>
<td>0.997</td>
<td>1.19</td>
<td>2.97</td>
</tr>
<tr>
<td>Mean aortic gradient</td>
<td>1.07(1.03-1.12)</td>
<td>0.90</td>
<td>1.19(0.97-1.47)</td>
<td>0.006</td>
</tr>
<tr>
<td>Valvuloarterial impedance</td>
<td>2.97(1.72-5.13)</td>
<td>.006</td>
<td>2.23 (1.26-3.94)</td>
<td>1.605(0.93-2.78)</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>.007</td>
<td>.006</td>
<td>.036</td>
<td>.039</td>
</tr>
</tbody>
</table>

95%CI, 95% confidence interval; Group I, normal flow and high gradient; Group II, normal flow and low gradient; Group III, low flow and high gradient; Group IV, low flow and low gradient; HR, hazard ratio (represents the increase in mortality per unit increase in the variable); ref., reference category.
similar prognosis to that of patients with moderate AS.In our study, as in previous publications, the surgery rate in patients with a low gradient was lower,10,14,22 possibly due to the clinical perception of less severe AS. Our results corroborate the finding that a low aortic gradient does not exclude the possibility of severe AS, as well as the benefits of surgery in these patients, regardless of the aortic gradient value.10,14,18,23–25

Clinical Implications

Our study shows that severe AS with low aortic flow results in a worse prognosis than severe AS with normal flow. Another important finding was the higher prevalence of AF in patients with low flow, which also has prognostic value.

This study confirms the benefits of valve replacement surgery in all symptomatic patients with severe stenosis (AVA<1 cm², according to current guidelines) regardless of aortic gradient. Importantly, most of the patients in this study had an AVA<0.8 cm², and only group II patients (low gradient and normal flow) tended to have an AVA>0.8 cm² (Table 2).

Group II had less severe stenosis and a better prognosis than the remaining groups in our study. Several publications on this topic have proposed a readjustment of the cut-off values for defining the severity of stenosis,13,22 suggesting that these patients in particular be classified as having moderate/severe stenosis.26 However, as our study has shown, this group also benefits from valve replacement surgery. This is a borderline group that is the subject of much debate. In these patients, careful clinical evaluation of symptoms and/or patient stratification using additional tests such as ergometry and aortic calcification score are essential.

Study Limitations

The primary limitation of our study is its retrospective design and the lack of randomization for surgical treatment, although all patients were referred to surgery due to the presence of symptoms following a medical/surgical evaluation.

The patients that did not undergo surgery formed a heterogeneous group. Surgery was not considered in many of these patients due to the presence of comorbidities that may not have been properly reflected in our study (such as advanced age, quality of life, and cognitive deterioration). In other patients, the symptoms may have derived from a noncardiac origin, or the AS may have been considered nonsevere. Some patients also refused surgery when offered. Nevertheless, we believe that these limitations reflect the reality of daily clinical practice and the management of patients with severe AS in our environment.

Finally, we would like to highlight the importance of correctly measuring aortic gradient (which may be underestimated in group II and IV patients) and SVI, and therefore, of careful measurement of the left ventricular outflow tract and velocity-time integral using Doppler echocardiography,27 which is even more important in patients with AF, given the different R-R interval.28 The retrospective design of our study implies that the values may have been obtained with these potential errors. However, we believe that the difference in prognosis between groups II and IV would rule out the possibility that this is an artificial calculation based on improperly calculated SV.

CONCLUSIONS

Our study demonstrates a worse prognosis of AS in the presence of low flow and normal EF. These results suggest that an analysis of aortic flow using Doppler echocardiography (SVI) may be useful for risk stratification and decision making on treatment in patients with AS.

CONFLICTS OF INTEREST

None declared.

REFERENCES


