

Clinical and Echocardiographic Aspects of Patient-Prosthesis Mismatch in Patients With Prosthetic Aortic Valves

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Abstract

Introduction: Patient-prosthesis mismatch (PPM) is considered to occur if a prosthetic heart valve has a high transvalvular pressure gradient and a reduced indexed valve area, despite normally functioning discs. PPM may have clinical and hemodynamic repercussions for patients.

Objective: To analyze the clinical and echocardiographic characteristics of PPM in patients with prosthetic aortic valves.

Methods: Patients aged 18 years or over with a biological or mechanical aortic valve undergoing follow-up since February 2010 were included. PPM was considered mild if the indexed valve area was $\geq 0.85 \text{ cm}^2/\text{m}^2$ and severe if $\leq 0.65 \text{ cm}^2/\text{m}^2$. Variables were compared between groups with moderate or severe PPM (PPMAO2) and mild PPM (PPMAO1); significant if $p < 0.05$.

Results: Sixty patients (36 women) with prosthetic aortic valves (29 biological and 31 mechanical) were included. PPMAO2 was diagnosed in 12 patients (20%), who had a mean valve area of $0.66 \text{ cm}^2/\text{m}^2$ and mean gradient of 24 mm Hg. Functional class II or III was more frequent in the PPMAO2 group (66.7%) than in the PPMAO1 group (20.8%); $p < 0.001$. Left atrial volume ($51 \pm 16 \text{ mL}/\text{m}^2 \times 40 \pm 12 \text{ mL}/\text{m}^2$; $p = 0.002$) and left ventricular septal and wall thicknesses ($10.83 \text{ mm} \times 10 \text{ mm}$; $p = 0.018$) were higher in the PPMAO2 group.

Conclusions: Moderate or severe PPM occurred in 20% of patients. These patients were more symptomatic and had higher left atrial volumes and left ventricular myocardial wall thickness.

Keywords: Aortic Valve; Heart Valve Prosthesis Implantation; Echocardiography.

Introduction

Approximately 90,000 prosthetic valves are implanted each year in the United States, with biological valves predominating over mechanical valves, but a marked increase has been recently observed in percutaneous bioprosthesis implantation worldwide.^{1,2} In Brazil, according to the Information Technology Department of the Brazilian Unified Health System (DATASUS for short, in Portuguese), 17.4% of major cardiovascular operations include prosthetic valve implantation.³ It is known that, after prosthetic valve implantation, patients with significant aortic stenosis progress with clinical and ventricular function improvement and increased survival.^{4,5}

Despite improvements in hemodynamic performance, durability, and surgical implantation techniques and reduction in thrombogenicity, prosthetic heart valves are still subject to complications or dysfunctions.⁶ Another condition that can occur with prosthetic heart valves is patient-prosthesis mismatch (PPM), which is characterized by high transvalvular pressure gradients and a reduced valve area indexed to the patient's body surface area, despite normally functioning discs.^{1,7} The diagnosis considers both the expected valve hemodynamic performance and the patient's cardiac output needs, largely related to body surface area.⁸ An imbalance between these two variables determines the high transvalvular pressure gradients that correspond to the initial expression of PPM.^{6,9}

PPM was first described and characterized in 1978 by Rahimtoola.¹⁰ Several studies have been developed with the purpose of better understanding hemodynamics and clinical consequences.¹¹⁻¹³ Currently, for the diagnosis of PPM in aortic valves, it is recommended that the effective valve area be calculated using Doppler echocardiography with the continuity equation.^{14,15} PPM has been associated with increased short- and long-term mortality after aortic valve replacement, reduced functional capacity, increased hospitalization for heart failure, and decreased prosthetic valve durability.¹⁶ In a meta-analysis of 34 studies including 27,186 patients and 133,141 patient-years with prosthetic

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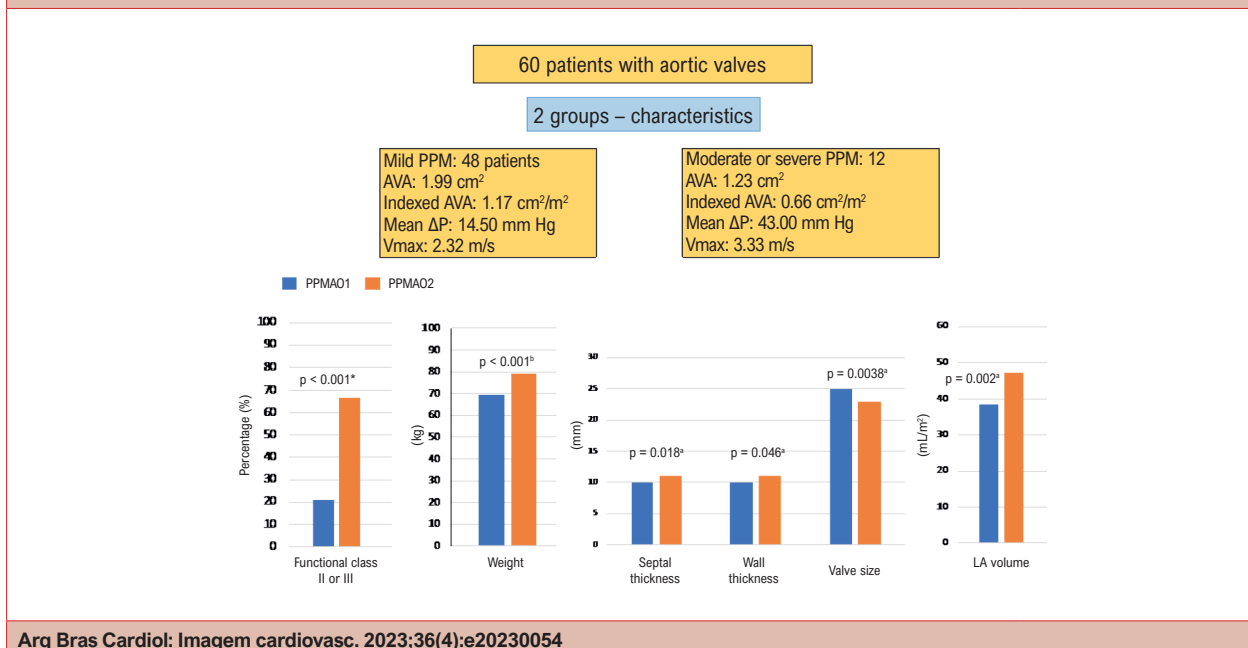
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Central Illustration: Clinical and Echocardiographic Aspects of Patient-Prosthesis Mismatch in Patients With Prosthetic Aortic Valves



The figure summarizes the hemodynamic characteristics of the patients' prosthetic valves (text boxes) and the main results of the study (bar graphs). The 60 patients were divided into two groups according to the hemodynamic pattern of the prosthetic aortic valves. One group with mild patient-prosthesis mismatch (PPMa01) and the other with moderate or severe patient-prosthesis mismatch (PPMa02); AVA: aortic valve area; ΔP: transvalvular systolic pressure gradient; Vmax: maximal transvalvular systolic velocity; LA: left atrium; p: statistical significance of the test; a: Mann-Whitney test; b: Student's t-test; *: Fisher's exact test. The values of the variables correspond to the mean or median depending on the normal or non-normal distribution of the variable and the test performed.

aortic valves, moderate and severe PPM increased all-cause mortality (hazard ratio, 1.19 and 1.84, respectively) and overall cardiovascular mortality (hazard ratio, 1.32 and 6.46, respectively).¹⁷

In Brazil, several valve types and designs are used in patients with severe valvular disease requiring surgery. There are imported prostheses, but there are also national prostheses, mainly biological valves. However, few studies in Brazil have addressed PPM.¹⁸ The 2020 guidelines for valvular heart diseases of the Brazilian Society of Cardiology¹⁹ mention PPM as a cause of prosthetic valve dysfunction, but they do not reliably and objectively describe the diagnostic aspects and possible measures to reduce or avoid its incidence.¹⁹ Conversely, a document published by the European Society of Cardiovascular Imaging, in collaboration with the Brazilian Department of Cardiovascular Imaging, suggests a flowchart similar to that of other international associations but does not detail the method to obtain the valve area for the diagnosis of PPM.²⁰

Therefore, the present study aimed to analyze the clinical and echocardiographic characteristics of PPM in a cohort of patients with prosthetic aortic valves.

Methods

Patients of either sex aged 18 years or over with a biological or mechanical aortic valve undergoing

outpatient follow-up since February 2010 were included, regardless of the number of previous operations. These patients had high transvalvular gradients previously defined by postoperative echocardiogram and had been attending regular outpatient follow-up visits with clinical and valve data available. Patients with previously confirmed prosthetic valve dysfunction or whose previous clinical and echocardiographic data did not allow the assessment of valve hemodynamics were excluded. The study was approved by the institution's Research Ethics Committee (CAAE: 65219317.4.0000.5505).

Clinical evaluation was performed during an outpatient visit along with a review of the patient's electronic or paper medical records if available. The following data were collected and recorded: sex; age; body surface area; body mass index (BMI); medical history, such as valvular disease leading to prosthetic valve implantation, hypertension, diabetes mellitus, and atrial fibrillation; and New York Heart Association (NYHA) functional class. The predicted effective valve area was obtained based on the type, brand, and size of the prosthetic valve reported in the patients' medical records, according to data from the literature or the manufacturer.

Doppler echocardiography

Echocardiography was performed using a Vivid 7 device (General Electric) equipped with 1- to 5-MHz transducers.

Simultaneous electrocardiogram derivation was used. The imaging planes used and the assessment of all 4 cardiac chambers, including left ventricular septal and posterior wall thickness, as well as the analysis of the systolic function parameters of the ventricles and native valves followed the recent recommendations of the American Society of Echocardiography.²¹

The prosthetic valves were analyzed based on morphological characteristics and leaflet or disc motion. The prosthetic valve area was calculated using the continuity equation and expressed as absolute values (cm²) and indexed values (cm²/m²). Maximal and mean pressure gradients and maximal flow velocity across the prosthetic valve were determined with continuous-wave Doppler. If present, prosthetic valve regurgitation was analyzed using the methods recommended in the guidelines of the American Society of Echocardiography.²² Inferior vena cava diameter (cm) and maximal tricuspid regurgitation velocity (m/s) were also analyzed.

PPM was suspected if the mean pressure gradient across the aortic valve on prior routine Doppler echocardiography was greater than 20 mm Hg in the absence of structural abnormalities or leaflet or disc motion and more than mild regurgitation. In patients with suspected PPM, another echocardiogram was performed to determine all the parameters needed to confirm or exclude the diagnosis. The diagnosis of PPM was based on the determination of the valve area indexed to the patient's body surface area (cm²/m²). PPM was considered mild if the indexed valve area was above 0.85 cm²/m², moderate if between 0.65 and 0.85 cm²/m², and severe if less than or equal to 0.65 cm²/m². For comparison purposes, patients were divided into two groups: PPMAO1 group, patients with mild PPM; and PPMAO2 group, patients with moderate or severe PPM.

Statistical analysis

The data were presented with descriptive analysis according to the normal or non-normal distribution of the variables. Statistical analysis was initially performed using summary measures: mean, median, minimum and maximum values, standard deviation (SD), and absolute and relative frequencies (percentage). Normally distributed continuous variables were presented as mean (SD), whereas non-normally distributed continuous variables were presented as median and interquartile range (IQR). The Shapiro-Wilk test was used to assess the normality of the data. The Mann-Whitney test, Student's t-test for independent samples, Pearson's chi-square test, and extension of Fisher's exact test were used for inferential analyses to compare the data according to the PPM classification.²³ The significance level was set at 5% for all comparisons. Statistical analyses were performed in SPSS version 24 and R version 3.6.3.²³

Results

Sixty patients with prosthetic aortic valves were included, 36 of whom were women (60%); 21 patients (35%) were

older than 60 years, 54 (90%) had hypertension, 12 (20%) had diabetes mellitus, and 8 (13%) had chronic kidney disease. According to the adopted criteria, moderate or severe PPM was diagnosed in 12 patients (20%) (PPMAO2 group). As expected, patients in the PPMAO2 group had significantly smaller absolute valve area and indexed valve area and significantly higher maximal transvalvular systolic velocity and maximal and mean transvalvular pressure gradients than those in the PPMAO1 group (Table 1).

At the time of the echocardiogram, the 12 patients in the PPMAO2 group had significantly higher weight, height, and BMI than those in the PPMAO1 group, but the two groups did not differ significantly in age or body surface area (Table 2).

The valve dysfunction before surgery or implantation underlying the indication for surgery was aortic stenosis in 46 (76.6%) patients, aortic insufficiency in 10 (16.7%), and double aortic valve dysfunction in 4 (6.6%). The cause of aortic valve dysfunction was degenerative (calcification) in 36 patients (60%), sequelae of rheumatic fever in 18 (30%), infective endocarditis in 4 (6.7%), and bicuspid aortic valve in 2 (3.3%). Among associated valvular diseases, 14 (23.3%) were mild mitral insufficiency, 4 (6.7%) were moderate mitral insufficiency (2 in the PPMAO2 group), and 18 (30%) were mild tricuspid insufficiency; 2 patients also had an ascending aorta tube graft that was implanted at the time of valve replacement surgery. There was no significant difference in the proportion of pure aortic stenosis before surgery between the PPMAO1 group (37 patients, 77.1%) and the PPMAO2 group (9 patients, 75.0%) ($p=0.856$; Fisher's exact test). Seven patients (11.7%) had a prosthetic valve in the mitral position; 2 of them were in the PPMAO2 group.

Of the 60 prosthetic aortic valves, 31 were mechanical and 29 were biological, 1 of which was implanted percutaneously. There was no significant difference in the proportion of mechanical valves between the groups; there were 27 mechanical valves (56.3%) in the PPMAO1 group and 8 (66%) in the PPMAO2 group ($p=0.552$; Fisher's exact test). There was also no significant difference in the proportion of biological valves between the PPMAO1 (41.4%) and PPMAO2 (33.3%) groups ($p=0.651$; Fisher's exact test). Regardless of prosthetic valve type, the size of the valves implanted in patients in the PPMAO2 group (median: 23 mm; IQR: 23.4-26.7 mm) was significantly smaller ($p=0.0038$; Mann-Whitney test) than that in patients in the PPMAO1 group (median: 25 mm; IQR: 22.2-24.7 mm). The median predicted effective valve area was 1.68 cm² (IQR: 1.79-2.08 cm²) in the PPMAO2 group and 2.03 cm² (IQR: 1.54-1.89 cm²) in the PPMAO1 group ($p=0.0013$; Mann-Whitney test).

NYHA functional class was I in 42 patients (70%), II in 13 (21.7%), and III in 5 (8.3%); no patient was in functional class IV. The proportion of patients with NYHA functional class II or III in the PPMAO2 group (8 patients; 66.7%) was significantly higher than that in the PPMAO1 group (10 patients; 20.8%) ($p<0.001$; Fisher's exact test). Atrial fibrillation was present in 7 patients (14.6%) in the PPMAO1 group and in 2 patients (16.7%) in the PPMAO2 group ($p=0.562$; Fisher's exact test).

Table 1 – Comparison of hemodynamic measurements in prosthetic aortic valves between patients with patient-prosthesis mismatch (PPM) classified as mild (PPMA01) and moderate or severe (PPMA02)

MEASUREMENT		PPMA01	PPMA02	p
	N	48	12	
PAV area (cm ²)	Mean ± SD	1.99 ± 0.32	1.23 ± 0.34	<0.001 ^b
Indexed PAV area (cm ² /m ²)	Mean ± SD	1.17 ± 0.21	0.66 ± 0.18	<0.001 ^b
Maximal ΔP (mm Hg)	Median	27.00	43.00	
	IQR	23.4 – 47.2	23.6 – 51.1	0.001 ^a
Mean ΔP (mm Hg)	Median	14.50	23.50	0.002 ^a
	IQR	9.4 – 31.2	16.4 – 31.4	
PAV Vmax (m/s)	Median	2.32	3.33	<0.001 ^a
	IQR	1.46 – 2.53	1.46–3.84	

^aMann-Whitney test; ^bStudent's t-test for independent samples; ΔP: pressure gradient; N: number of patients (the same for all measurements); IQR: interquartile range; p: p-value of statistical comparison between groups; PAV: prosthetic aortic valve; Vmax: maximal flow velocity.

Table 2 – Anthropometric characteristics of patients with patient-prosthesis mismatch (PPM) classified as mild (PPMA01) and moderate or severe (PPMA02) in prosthetic aortic valves

Variable		PPMA01	PPMA02	p
	N	48	12	
Age (years)	Median	60.23	66.51	0.456 ^a
	IQR	40 – 74.5	47.4–82.4	
Weight (kg)	Mean ± SD	69.45 ± 4.78	79.23 ± 8.91	<0.001 ^b
BMI (kg/m ²)	Median	26.71	31.45	<0.001 ^a
	IQR	30.14 – 32.8	31.4 – 35.6	
BSA (m ²)	Median	1.75	1.89	0.056 ^a
	IQR	1.73 – 1.91	1.98 – 2.08	
Height (cm)	Mean ± SD	176 ± 6.45	181 ± 5.78	0.049 ^b

Mann-Whitney test; ^bStudent's t-test for independent samples; BSA: body surface area; BMI: body mass index; IQR: interquartile range; N: number of patients (the same for all variables); p: p-value of statistical comparison; SD: standard deviation.

The indexed left atrial volume was significantly higher in patients with moderate or severe PPM than in those with mild PPM. There was no significant difference in the values of end-diastolic and end-systolic diameters and of left ventricular mass index and ejection fraction between the two groups, but myocardial wall thickness was significantly higher in patients in the PPMA02 group than in the PPMA01 group (Table 3). Measurements of the right chambers were not significantly different between the two groups (Table 3). Maximal tricuspid regurgitation velocity was not significantly different between patients in the PPMA01 group (mean: 2.61 m/s; SD: 0.48 m/s) and those in the PPMA02 group (mean: 2.38 m/s; SD: 0.48 m/s). The Central Figure summarizes the main results of the study.

Discussion

The present study analyzed PPM, clinical impairment, and structural and functional features on echocardiography in patients with prosthetic aortic valves. As expected,

due to the division of patients, there was a significant difference in valve area, indexed valve area, transvalvular gradients, and maximal systolic velocity between the two groups. The values of these variables in the present study are consistent with those reported in previous studies. The mean maximal transvalvular systolic velocity in the present study was 3.21 m/s, higher than that in the study conducted by Zorn et al, who found a value of 2.88 m/s in their sample.²⁴ The mean gradient of patients with moderate or severe PPM in the present study was 24.2 mm Hg, similar to that found by Echahidi et al, who described a mean of 25 mm Hg in patients with severe PPM; in the same study, in patients with moderate PPM, the mean gradient was 19 mm Hg.²⁵ In the study by Otto et al, the mean gradient was 28 mm Hg in patients with severe aortic PPM and 21 mm Hg in patients with moderate PPM.¹⁸

PPM was observed in 20% of the prosthetic aortic valves in the present study. Similar findings were observed in previous studies, such as those by Dayan et al and

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Bonderman et al, who also observed 20% of PPM in patients with prosthetic aortic valves.^{26,27} Kohsaka et al found 11% of severe PPM in a sample of 469 patients; it is worth noting that PPM was mainly found in mechanical aortic valves of sizes 19 to 23.²⁸ The rate of PPM diagnoses could decrease with reclassification based on patients' BMI, but this has not been observed in most published studies.²⁰ It is worth noting that 8 patients in the PPMAO2 group had a BMI greater than 30 mg/kg². This variable was significantly higher in the PPMAO2 group, but the aim of the study was not to analyze this other classification, but rather the preestablished criteria for determining PPM and its severity.

In the present study, PPM was diagnosed based on the measurement of the effective valve area by Doppler echocardiography corrected for body surface area in patients with prosthetic aortic valves without evidence of dysfunction. Although the absolute value of the valve area can be used, the valve area indexed to the patient's body surface area appears to have greater sensitivity and specificity as reported by Hanyama et al.²⁹

The mean indexed valve area in patients with moderate or severe aortic PPM was 0.66 cm²/m². Echahidi et al found

an indexed valve area of 0.53 cm²/m² in patients with severe PPM and 0.73 cm²/m² in patients with moderate PPM.²⁵ Zorn et al identified a mean valve area of 0.42 cm²/m² in patients with severe PPM.²⁴ Otto et al identified a mean of 0.51 cm²/m² in patients with severe PPM and 0.73 cm²/m² in patients with moderate PPM.¹⁸ The indexed valve area in the present study was, therefore, slightly higher than that reported in previous studies, possibly due to the inclusion of moderate and severe PPM in the same group.

The main parameters that determine PPM are the effective valve area and the patient's need for greater cardiac output, usually related to the patient's size. In the present study, there was no difference in the proportion of mechanical and biological valves between the two groups. However, both the size of the valves used and the effective valve area predicted before surgery were significantly smaller in patients with moderate or severe PPM than in those with mild PPM. Body surface area was not significantly different between the groups. Considering that the diagnosis of PPM is based on the effective valve area indexed to the patient's body surface area, this finding differs from that of previous studies reporting that patients with severe PPM have a greater body surface area.^{30,31}

Table 3 – Comparison of echocardiographic measurements in left and right chambers between patients with patient-prosthesis mismatch (PPM) classified as mild (PPMAO1) and moderate or severe (PPMAO2) in prosthetic aortic valves

MEASUREMENT		PPMAO1	PPMAO2	p
	N	48	12	
iLAV (mL/m ²)	Median	38.40	47.40	0.002 ^a
	IQR	29.6 – 84.5	43.4 – 87.6	
LVDd (mm)	Mean ± SD	50.42 ± 5.95	52.08 ± 5.16	0.378 ^b
LVDs (mm)	Median	32.00	34.00	0.394 ^a
	IQR	29.5 – 51.4	29.4 – 41.4	
IVST (mm)	Median	10.00	11.00	0.018 ^a
	IQR	9 – 14	9 – 13	
WT (mm)	Median	10.00	11.00	0.046 ^a
	IQR	10 – 12	8 – 12	
MI (g/m ²)	Mean ± SD	103.98 ± 24.89	121.33 ± 37.91	0.058 ^b
LVEF (%)	Median	60.00	56.00	0.111 ^a
	IQR	35 – 61.4	41.4 – 58.7	
iRAV (mL/m ²)	Median	20.00	22.00	0.236 ^a
	IQR	19.8 – 31.7	17.8 – 33.2	
RVDd (mm)	Median	23.00	25.50	0.009 ^a
	IQR	21.2 – 30.6	24.5 – 31.4	
PCRVA (%)	Mean ± SD	42.96 ± 8.42	46.57 ± 6.72	0.175 ^b
TAPSE (mm)	Mean ± SD	19.50 ± 2.90	20.17 ± 2.66	0.472 ^b
TAV (cm/s)	Mean ± SD	11.16 ± 1.57	11.26 ± 1.49	0.846 ^b

^aMann-Whitney test, ^bStudent's t-test for independent samples. Dd: end-diastolic diameter; Ds: end-systolic diameter; WT: wall thickness; IVST: interventricular septal thickness; TAPSE: tricuspid annular plane systolic excursion; EF: ejection fraction; MI: mass index; IQR: interquartile range; N: number of patients (the same for all analyses); p: p-value of statistical comparison; iRAV: indexed right atrial volume; iLAV: indexed left atrial volume; RV: right ventricle; LV: left ventricle; TAV: tricuspid annular velocity; PCRVA: percentage change in right ventricular area; LVEF: Left ventricular ejection fraction.

It is possible that the smaller size of the valves implanted in patients with moderate or severe PPM may have had a greater impact on the diagnosis of PPM. However, as the comparison of body surface area had a p-value of 0.056 and weight, height and BMI were significantly higher in the group with moderate or severe PPM, a possible impact of this variable on the rate of diagnoses cannot be ruled out.

Rheumatic disease as the cause of native valve disease leading to valve replacement had a considerable proportion of 30%, but less than that of degenerative cause. In the study by Otto et al, the prevalence of rheumatic disease in patients with severe PPM was 43.1%, while in the Brazilian study by Oliveira et al it was 29.7%.^{3,18} The incidence of rheumatic disease remains high in Brazil, so a high proportion in relation to degenerative disease was expected compared with studies conducted in high-income countries, as shown by Mothy et al.³²

The proportion of NYHA functional class II or III was significantly higher in patients with moderate or severe PPM than in those with mild PPM. These data contrast with those described by Vicchio et al, who administered a questionnaire to patients with PPM but did not identify a significant difference in functional class in those with severe PPM.³³ Atrial fibrillation was uncommon in the present study, with no significant difference in its proportion between the groups.

Patients with moderate or severe PPM had significantly higher left ventricular septal and myocardial wall thicknesses than those with mild PPM. However, this was not accompanied by a significant difference in the left ventricular mass index. The left ventricular mass index is the mass calculated based on left ventricular wall thicknesses and end-diastolic diameter. The value is corrected for body surface area and defines the existence of ventricular hypertrophy on echocardiogram. Although we cannot state that patients with moderate to severe PPM had left ventricular hypertrophy, the greater myocardial wall thickness suggests a possible consequence.

The absence of a difference in left ventricular end-diastolic and end-systolic diameters between the two patient groups in the present study was similar to that of the study by Zorn et al.²⁴ The indexed left atrial volume was higher in patients with moderate or severe PPM. A contributing factor could be the association of mitral valve disease prior to aortic valve surgery. However, 3 of the 7 patients with a prosthetic valve in the mitral position due to significant previous mitral valve disease were in the PPMAO2 group, whereas 2 of the 4 patients with moderate mitral insufficiency were in the PPMAO2 group. Another contributing factor could be left ventricular diastolic dysfunction as a result of the greater pressure gradient in the prosthetic aortic valve and the greater myocardial wall thickness. However, left ventricular diastolic function was not analyzed in the present study.

This study has limitations that need to be addressed. As it was a retrospective cohort analysis, the echocardiograms that defined PPM were performed late in relation to the time of surgery. To better characterize PPM, echocardiograms

should have been performed a few weeks after patients were discharged from the hospital. Some patients in the study had transvalvular systolic velocity greater than 3.0 m/s. The criteria recommended by the 2009 American Society of Echocardiography guidelines could have been applied to this group,²³ which include measurement of acceleration time and analysis of the contour of the velocity curve of continuous-wave Doppler. This analysis was not included in the present study, because patients with a possible diagnosis of prosthetic valve dysfunction had already been excluded from the study. The relatively small sample size was also a limitation.

Conclusion

The rate of moderate or severe PPM in aortic valves was 20%. There was no impact of valve type (biological or mechanical) on the proportion of PPM, but valve size and the effective valve area predicted before surgery were smaller in patients with moderate or severe PPM and may have been determining factors.

Patients with moderate or severe PPM were more symptomatic than those with mild PPM, despite no significant differences in the proportion of atrial fibrillation or left ventricular ejection fraction. The indexed left atrial volume and left ventricular myocardial wall thickness were the echocardiographic variables with significantly higher values in patients with moderate or severe PPM.

Author Contributions

Conception and design of the research: Bispo IGA, Hemery DFA, Kyiose AT, Moises VA; Acquisition of data, analysis and interpretation of the data, statistical analysis and writing of the manuscript: Bispo IGA, Moises VA; critical revision of the manuscript for intellectual content: Bispo IGA, Hemery DFA, Fischer CH, Moises VA.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Universidade Federal de São Paulo under the protocol number 20/4820 CAAE 65211317400005505. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

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