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Valvular Heart Disease

From Basic to Clinical Advances

Edited by
Rihab Bouchareb

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Valvular Heart Disease: From Basic to Clinical Advances

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Guest Editor

Rihab Bouchareb

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Guest Editor
Rihab Bouchareb
Icahn School of Medicine at
Mount Sinai
New York, USA

Editorial Office
MDPI AG
Grosspeteranlage 5
4052 Basel, Switzerland

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Article

Functional Mitral Regurgitation Post-Isolated Aortic Valve Replacement

Petar Dabic [1], Bojan Vucurevic [1], Milorad Sevkovic [1], Dusan Andric [2], Slobodan Pesic [3], Mihailo Neskovic [3,4], Sasa Borovic [4,5] and Jovan Petrovic [1,*]

[1] Department of Cardiology and Internal Medicine, Vascular Surgery Clinic, Institute for Cardiovascular Diseases Dedinje, 11000 Belgrade, Serbia
[2] Department of Cardiology, University Children's Hospital, 11000 Belgrade, Serbia
[3] Vascular Surgery Clinic, Institute for Cardiovascular Diseases Dedinje, 11000 Belgrade, Serbia
[4] Faculty of Medicine, University of Belgrade, 11000 Belgrade, Serbia; sborovic@ikvbd.com
[5] Heart Failure Center, Institute for Cardiovascular Diseases Dedinje, 11000 Belgrade, Serbia
* Correspondence: jovan.petrovic@institutdedinje.org

Abstract: Background: The management of mitral regurgitation during aortic valve replacement remains a complex question. Secondary mitral regurgitation often improves post-aortic valve replacement without mitral valve surgery, but residual mitral regurgitation can significantly affect long-term outcomes. This study investigates the natural history of mitral regurgitation following isolated aortic valve replacement and identifies prognostic factors for persistent mitral regurgitation. **Methods**: A retrospective study was conducted on 108 patients who underwent isolated aortic valve replacement. Patients were categorized based on mitral regurgitation improvement. Additionally, patients were divided into patient-prosthesis mismatch and non-patient-prosthesis mismatch groups based on the aortic prosthesis. Preoperative and postoperative echocardiographic data were analyzed. **Results**: In total, 63% of patients showed mitral regurgitation improvement. The improved functional MR group showed significant reductions in peak and mean transvalvular pressure gradients. In contrast, the patient-prosthesis mismatch group had persistent mitral regurgitation improvement in 59.2% of patients. The non-patient-prosthesis mismatch group exhibited significant structural improvements and a reduction in mitral regurgitation severity in 68.6% of patients. **Conclusions**: The study shows that aortic valve replacement could significantly improve MR when patient-prosthesis mismatch is avoided. This approach maximizes hemodynamic outcomes, mitigates the risk of residual or worsening mitral regurgitation, and potentially reduces the need for additional mitral valve interventions.

Keywords: patient-prosthesis mismatch; aortic valve replacement; mitral regurgitation; aortic stenosis

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1. Introduction

Mitral regurgitation (MR) accompanies severe aortic valve stenosis (AS). Around 75% of patients undergoing aortic valve replacement (AVR) for AS have some degree of MR [1,2]. The management of MR in patients undergoing AVR remains a complex and debated area in cardiovascular surgery, particularly when considering whether to perform concomitant MV interventions. The variability in guidelines reflects differing interpretations of the available evidence and the challenges of balancing potential benefits against increased surgical risks [3,4].

Surgical intervention on the mitral valve (MV) is generally unnecessary when there are no leaflet abnormalities, annulus distention, or significant left ventricular (LV) geometry issues. Additionally, non-severe secondary MR frequently betters following aortic valve treatment [3]. While MV surgery during AVR increases perioperative complications, the

effect of residual MR on survival, quality of life, and myocardial remodeling is significant. Perioperative mortality rates following AVR are around 2.2%. However, in patients undergoing both AVR and MV replacement, these rates rise to 9% [4]. Several studies suggest that MV repair during double valve surgery might be advantageous compared to MV replacement, potentially reducing long-term risk without a corresponding rise in perioperative mortality [5–7]. According to some studies, patients whose MR improved postoperatively had significantly higher 5-year survival rates compared to those without MR improvement [8,9].

AVR for severe AS is designed to decrease LV afterload, potentially initiating reverse remodeling of the LV. These changes are anticipated to positively influence MV mechanics, potentially resolving secondary MR that arises without structural abnormalities of the mitral apparatus. However, the expected improvement in MR is often not realized.

This study aimed to investigate the natural progression of functional MR (FMR) following AVR and identify echocardiographic parameters linked to persistent MR.

2. Materials and Methods

In continuation of our earlier published research, we conducted a case-control study with an expanded sample size [10]. Over 16 years, 3014 patients underwent isolated AVR for severe AS in our tertiary care center. We included adult patients, aged 18 and older, who had undergone aortic valve replacement with either a bileaflet mechanical or bioprosthetic valve and who had FMR. Eligible patients required postoperative echocardiographic data at both discharge and at a 6-month follow-up to assess outcomes over time. Only patients with stable postoperative conditions, free from complications necessitating additional surgery were included. Preoperative echocardiographic data, such as left ventricular outflow tract diameter and left ventricular ejection fraction, were required for baseline comparisons. Exclusion criteria included morphological mitral apparatus abnormalities, chordal rupture, leaflet calcification, fibrosis or prolapse, coronary artery disease, aortic disease, previous open-heart procedures, and congenital heart abnormalities. Ultimately, 108 patients were included in our study, which were analyzed retrospectively. AVR was performed using St. Jude Medical™ Hemodynamic Plus Aortic Valve and St. Jude Medical™ Biocor™ Pericardial Stented Tissue Valve.

Patients were categorized into two groups based on the presence of improvement in their FMR post-AVR. The Persistent FMR group maintained a moderate to moderate-severe grade (2+ and 3+) of MR after AVR. The Improved FMR group showed a reduction in MR grade (less than 2+) following AVR. The MR severity was based on the measurement of vena contracta. Additionally, patients were divided based on the indexed Effective Orifice Area (EOAi) of the implanted aortic prosthesis. The EOA was calculated using the manufacturer's published values, which were then indexed to the body surface area. The Prosthesis-Patient Mismatch group (PPM) included patients with an EOAi $\leq 0.85\ cm^2/m^2$, and the Non-Prosthesis-Patient Mismatch group (non-PPM) included patients with an EOAi $> 0.85\ cm^2/m^2$.

All patients included in the study underwent preoperative and postoperative transthoracic echocardiography. Postoperative echocardiography was routinely performed at discharge and again at the 6-month follow-up. For analysis, we used data from the 6-month follow-up assessment. The echocardiographic analysis included a comprehensive assessment using M-mode, two-dimensional imaging, and Doppler echocardiography protocols as per the guidelines set forth by the European Association of Cardiovascular Imaging and the American Society of Echocardiography [11–13]. All patients provided written informed consent for the publication of their study data.

Statistical Analysis

We conducted data analysis using parametric or nonparametric methods based on the nature of the variables. Descriptive statistics were used to express observed characteristics, including mean values with standard deviation for normally distributed data, and median

with interquartile range for non-normally distributed data. For continuous nonparametric data, Wilcoxon's signed-rank test was employed, whereas continuous parametric data were analyzed using Student's *t*-test and paired *t*-test as appropriate. Categorical data were analyzed using the Chi-square test or Fisher's exact test to determine statistically significant differences between groups. The significance level was set at 2-sided $p < 0.05$. The statistical analysis was performed using SPSS Statistics 26 (IBM, Armonk, NY, USA).

3. Results

We observed that 68 patients (62.9%) showed improvement in FMR postoperatively, while 40 patients (37.1%) had persistent FMR after the procedure. There were no significant differences in preoperative parameters, except for the value of left ventricular ejection fraction (LVEF) between persistent and improved FMR groups (40.6 ± 18.1% vs. 54.3 ± 12.9%, $p = 0.028$) (Table 1).

Table 1. Patient characteristics according to postoperative mitral regurgitation.

	Persistent FMR (*n* = 40)	Improved FMR (*n* = 68)	*p*-Value
Age (years)	58.5 ± 12.8	63.1 ± 11.2	0.053
Male sex (*n*, %)	23 (57.5)	28 (41.2)	0.150
BMI (kg/m^2)	26.9 ± 3.9	26.7 ± 4.4	0.412
AF	3 (7.5)	8 (11.7)	0.743
NYHA class			
II	37 (92.5)	65 (95.6)	1.000
III	3 (7.5)	3 (4.4)	
TAV	38 (95.0)	63 (92.6)	1.000
AVA (cm^2)	0.7 ± 0.1	0.6 ± 0.2	0.127
Peak gradient (mmHg)	91.1 ± 30.7	107.1 ± 31.2	0.061
Mean gradient (mmHg)	55.7 ± 21.2	71.6 ± 24.3	0.059
LVEDD (mm)	56.9 ± 12.5	52.3 ± 6.1	0.093
LVESD (mm)	42.3 ± 12.5	36.3 ± 7.2	0.055
Septum thickness (mm)	12.1 ± 1.8	12.7 ± 1.7	0.723
Posterior wall thickness (mm)	11.9 ± 1.6	11.7 ± 1.5	0.785
LA (mm)	42.3 ± 4.5	43.2 ± 4.7	0.436
LVEF (%)	40.6 ± 18.1	54.3 ± 12.9	0.028
TR grade			
0	22 (55)	37 (54.4)	1.000
I	8 (20)	14 (20.6)	
II	10 (25)	17 (25)	
RVSP (mmHg)	46.4 ± 8.3	44.1 ± 11.2	0.517
EOAi (cm^2/m^2)	1.3 ± 0.4	1.2 ± 0.1	0.799

AF—atrial fibrillation; AVA—aortic valve area; BMI—body mass index; EOAi—indexed Effective Orifice Area; FMR—functional mitral regurgitation; LA—left atrium; LVEDD—left ventricle end-diastolic diameter; LVEF—left ventricle ejection fraction; LVESD—left ventricle end-systolic diameter; RVSP—right ventricle systolic pressure; TAV—tricuspid aortic valve; TR—tricuspid regurgitation.

A significant reverse remodeling of the LV was observed evidenced by reductions in LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), LV posterior wall and septum thickness (Table 2). These findings collectively indicate favorable changes in LV structure and function following AVR in patients with improved FMR.

The PPM group included 22 patients (20.4%) and the non-PPM included 86 patients (79.6%) (Table 3). The average EOAi for the PPM group was 0.72 ± 0.61 and for non-PPM 1.11 ± 0.20 cm^2/m^2. In the PPM group following AVR, there was a notable decrease in transvalvular pressure gradients. Despite these reductions, other echocardiographic parameters remained largely unchanged. Notably, MR grade persisted at ≥2+ in 59.2% of the patients, indicating that while AVR effectively reduced pressure gradients, it did not uniformly improve MR severity in patients with PPM (Table 4). Conversely, in the non-PPM group, following AVR, significant reductions were also observed in both transvalvular pressure gradients (Table 4). Additionally, significant reverse remodeling of the LV occurred,

evidenced by reductions in LVEDD, LVESD, septum thickness, and LV posterior wall thickness. This indicates a favorable structural and functional adaptation of the LV in this group. Furthermore, a substantial improvement in MR was noted, with the MR grade reducing below 2+ in most patients (68.6%) (Table 4). This underscores the more consistent and beneficial effects of AVR in the non-PPM group in terms of both pressure gradient reduction and MV function.

Table 2. Echocardiographic parameters of patients according to postoperative mitral regurgitation.

	Persistent FMR (*n* = 40)			**Improved FMR (*n* = 68)**		
	Preoperative	**Postoperative**	***p*-Value**	**Preoperative**	**Postoperative**	***p*-Value**
Peak gradient (mmHg)	91.1 ± 30.7	29.3 ± 9.1	0.002	107.1 ± 31.2	28.1 ± 7.23	0.001
Mean gradient (mmHg)	55.7 ± 21.2	15.8 ± 5.4	0.001	71.6 ± 24.3	14.6 ± 4.5	0.003
LVEDD (mm)	56.9 ± 12.5	57.7 ± 11.9	0.912	52.3 ± 6.1	50.1 ± 3.2	0.021
LVESD (mm)	42.3 ± 12.5	41.1 ± 13.6	0.712	36.3 ± 7.2	32.2 ± 5.3	0.010
Septum thickness (mm)	12.1 ± 1.8	11.7 ± 2.1	0.373	12.7 ± 1.7	10.9 ± 1.9	0.004
Posterior wall thickness (mm)	11.9 ± 1.6	12.2 ± 1.5	0.223	11.7 ± 1.5	10.7 ± 1.2	0.022
LA (mm)	42.3 ± 4.5	42.3 ± 4.8	0.577	43.2 ± 4.7	42.1 ± 4.1	0.059
LVEF (%)	40.6 ± 18.1	41.8 ± 15.9	0.844	54.3 ± 12.9	57.4 ± 7.5	0.193
TR grade						
0	22 (55)	19 (47.5)		37 (54.4)	34 (50)	
I	8 (20)	6 (15)	0.527	14 (20.6)	22 (32.4)	0.527
II	10 (25)	15 (37.5)		17 (25)	11 (16.1)	
III	0	0		0	1 (1.5)	
RVSP (mmHg)	46.4 ± 8.3	40.8 ± 9.1	0.589	44.1 ± 11.2	39.9 ± 3.2	0.248

AVR—aortic valve replacement; FMR—functional mitral regurgitation; LA—left atrium; LVEDD—left ventricle end-diastolic diameter; LVEF—left ventricle ejection fraction; LVESD—left ventricle end-systolic diameter; RVSP—right ventricle systolic pressure; TR—tricuspid regurgitation.

Table 3. Preoperative patients' characteristics according to Prosthesis-Patient Mismatch.

	PPM (*n* = 22)	**Non-PPM (*n* = 86)**	***p*-Value**
Age (years)	70.1 ± 5.9	68.2 ± 7.1	0.079
Male sex (*n*, %)	6 (27.3)	38 (44.2)	0.224
BMI (kg/m^2)	25.7 ± 3.9	28.7 ± 5.5	0.681
AF	5 (22.7)	13 (15.1)	0.521
NYHA class			
II	19 (86.7)	81 (94.2)	0.356
III	3 (13.3)	5 (5.8)	
TAV	21 (95.5)	72 (83.7)	0.297
AVA (cm^2)	0.7 ± 0.4	0.7 ± 0.2	0.513
Peak gradient (mmHg)	89.7 ± 23.1	107.1 ± 30.2	0.472
Mean gradient (mmHg)	55.6 ± 20.1	67.2 ± 26.8	0.516
LVEDD (mm)	54.7 ± 5.2	53.9 ± 9.2	0.881
LVESD (mm)	37.6 ± 5.4	37.1 ± 11.1	0.934
Septum thickness (mm)	11.8 ± 1.9	12.2 ± 1.9	0.764
Posterior wall thickness (mm)	12.1 ± 1.1	11.9 ± 1.9	0.611
LA (mm)	43.3 ± 2.7	41.9 ± 5.3	0.799
LVEF (%)	49.9 ± 14.9	50.2 ± 16.1	0.862
TR grade			
0	16 (72.7)	45 (52.3)	0.064
I	6 (27.3)	27 (31.4)	
II	0	14 (16.3)	

AF—atrial fibrillation; AVA—aortic valve area; BMI—body mass index; LA—left atrium; LVEDD—left ventricle end-diastolic diameter; LVEF—left ventricle ejection fraction; LVESD—left ventricle end-systolic diameter; PPM—Prosthesis-Patient Mismatch; TAV—tricuspid aortic valve; TR—tricuspid regurgitation.

Table 4. Echocardiographic parameters of patients according to Prosthesis-Patient Mismatch.

	PPM (n = 22)			Non-PPM (n = 86)		
	Preoperative	**Postoperative**	***p*-Value**	**Preoperative**	**Postoperative**	***p*-Value**
Peak gradient (mmHg)	89.7 ± 23.1	32.9 ± 11.7	0.012	107.1 ± 30.2	28.3 ± 7.2	0.008
Mean gradient (mmHg)	55.6 ± 20.1	18.2 ± 7.1	0.027	67.2 ± 26.8	15.1 ± 4.3	0.002
LVEDD (mm)	54.7 ± 5.2	54.1 ± 4.9	0.675	53.9 ± 9.2	52.7 ± 8.8	0.032
LVESD (mm)	37.6 ± 5.4	36.7± 4.9	0.771	37.1 ± 11.1	35.1 ± 11.1	0.010
Septum thickness (mm)	11.8 ± 1.9	11.2 ± 1.5	0.473	12.2 ± 1.9	10.8 ± 1.5	0.001
Posterior wall thickness (mm)	12.1 ± 1.1	11.8± 0.9	0.464	11.9 ± 1.9	10.91 ± 1.49	0.001
LA (mm)	43.3 ± 2.7	42.7 ± 1.6	0.822	41.9 ± 5.3	41.8 ± 4.9	0.221
LVEF (%)	49.9 ± 14.9	51.5 ± 11.1	0.207	50.2 ± 16.1	51.9 ± 14.2	0.287
TR grade						
0	16 (72.7)	11 (50)	0.157	45 (52.3)	40 (46.5)	0.434
I	6 (27.3)	8 (36.4)		27 (31.4)	25 (29.1)	
II	0	3 (13.6)		14 (16.3)	19 (22.1)	
III	0	0		0	2 (2.3)	
MR grade						
≥2+	22 (100)	13 (59.2)	0.002	86 (100)	27 (31.4)	0.000
<2+	0	9 (40.8)		0	59 (68.6)	

AVR—aortic valve replacement; LA—left atrium; LVEDD—left ventricle end-diastolic diameter; LVEF—left ventricle ejection fraction; LVESD—left ventricle end-systolic diameter; MR—mitral regurgitation; PPM—Prosthesis-Patient Mismatch; TR—tricuspid regurgitation.

4. Discussion

The data collected from our study provide critical insights into the differential impacts of AVR on patients with and without PPM. The impact of AVR on MR and other echocardiographic parameters shows notable differences between the PPM and non-PPM groups.

These differences may be due to several factors. First, the persistence of MR post-AVR suggests that factors other than LV afterload may play a role in the pathophysiology of MR in these patients. For instance, the duration and severity of AS before AVR might lead to irreversible changes in the LV that continue to affect MV function even after the correction of AS. Moreover, the presence of PPM is another critical variable influencing outcomes. As observed in our study, patients with PPM showed less improvement in MR post-AVR compared to those without mismatch. This suggests that optimal prosthesis sizing and selection are crucial for maximizing the therapeutic benefits of AVR, including the mitigation of secondary MR.

Barreiro et al. observed significant postoperative improvements in MR, with 82% of patients showing resolution [9]. This high rate of improvement underscores the potential for significant cardiac function recovery when left ventricular afterload is reduced by AVR. Similarly, Vanden Eynden et al. highlighted the predictive role of preoperative factors, noting that isolated ischemic and functional MR were significant predictors of MR improvement post-AVR [14]. This insight is particularly valuable as it suggests that the nature of MR, whether ischemic or functional, can influence the therapeutic outcomes of AVR, emphasizing the need for a nuanced preoperative assessment. In our study, the improvement rate of FMR postoperatively was 62.9%. While this rate is somewhat lower than that reported by Barreiro et al., it nonetheless represents a substantial proportion of patients experiencing beneficial changes in mitral valve function following AVR. The disparity in improvement rates may be attributed to differences in patient populations, severity of preoperative MR, or the presence of factors such as PPM, which we found to influence postoperative outcomes.

On the other hand, Asher et al. demonstrated that MV repair or replacement for more-than-moderate MR at the time of CABG may be reasonable in a suitably selected CABG population but not for AVR, with or without coronary artery bypass grafting [15].

Harling et al. showed that the structural remodeling caused by severe AS regresses after AVR, as evidenced by reductions in LV mass and end-diastolic diameter. [16]. Some

studies identified factors associated with LV remodeling, such as higher preoperative LV mass, larger LV diastolic diameter and end-diastolic volume as independent predictors of improvement in MR after aortic valve surgery. These studies suggest that when there is potential for reverse remodeling, a more substantial improvement in MR is likely to occur after AVR [17,18]. Our study demonstrated an association between improvements in FMR and markers of LV remodeling, as shown by reductions in LVEDD, LVESD, and septal and LV posterior wall thickness. However, we recognize that our findings are associative and do not establish a causal relationship between FMR improvement and LV remodeling.

The findings of Harling et al. and subsequent research underscore a key physiological insight: the structural changes in the heart due to severe AS are not permanent and can be partially reversed following AVR. This process of reverse remodeling includes reductions in LV mass, LVEDD, and other structural dimensions, which are vital for improving cardiac function and patient outcomes [16–18]. A reduction in the size and mass of the LV generally leads to less tension on the MV apparatus, thereby improving leaflet coaptation and reducing regurgitation. Additionally, a decrease in the pressure and volume overload in the LV due to improved valve function helps in normalizing the dimensions and functioning of the heart, which contributes to the alleviation of MR [19].

Several studies identified factors associated with the progression of MR [20,21]. These included left atrial growth, atrial fibrillation, LV dysfunction, peak AV gradient < 60 mmHg, increased LV mass, elevated tricuspid regurgitation (TR) velocity, and elevated LV mass. In contrast, Joo et al. reported that increased right ventricular systolic pressure was the only significant predictor of postoperative MR [22]. Unger et al. found that postoperative MR was more likely to improve in patients with reduced LVEF and increased LV size [17]. Jeong et al. demonstrated that patients with preoperative atrial fibrillation and an LVEF > 40% were more likely to have residual MR [23]. Additionally, Sehovic et al. found that LVEDD > 54 mm, effective regurgitant orifice area > 25 mm^2, regurgitation volume > 40 mL/beat, pulmonary artery systolic pressure > 40 mmHg, and LA diameter > 45 mm were factors contributing to worsening MR [24]. PPM and low LVEF are both significant predictors of limited FMR recovery post-AVR.

In patients with moderate MR undergoing AVR, particularly those with low LVEF, we recognize that limited improvement in LVEF and the potential for ongoing LV and annular dilatation may restrict LV remodeling and FMR improvement. This raises a clinically relevant consideration for concomitant MVR. Moreover, PPM can place an additional and continuous load on the LV, which could impede LV remodeling and FMR recovery.

The findings in our study were the significant differences in postoperative pressure gradients between the persistent and improved FMR groups. Specifically, we observed substantial reductions in both peak and mean transvalvular gradients postoperatively. In the persistent FMR group, the postoperative peak gradient reduction was 61.8 mmHg ($p = 0.002$), and the mean gradient reduction was 39.9 mmHg ($p = 0.001$). In contrast, the improved FMR group demonstrated even greater reductions, with a postoperative peak gradient reduction of 79 mmHg ($p = 0.001$) and a mean gradient reduction of 57 mmHg ($p = 0.003$). These findings indicate a robust reduction in transvalvular gradients particularly in the improved FMR group. The lower preoperative values of LVEF observed in the persistent FMR group may partially explain this phenomenon, suggesting a "low flow–low gradient" effect. In this scenario, compromised LV function before surgery can lead to lower flow rates across the aortic valve, resulting in less significant pressure gradients even after AVR.

Previous studies have highlighted the negative impact of more than mild PPM, defined as an EOAi ≤ 0.85 cm^2/m^2, on various outcomes following AVR. These outcomes include less symptomatic improvement, worse hemodynamics at rest and during exercise, reduced regression of LV hypertrophy, and increased cardiac events postoperatively [25]. In our study, we specifically investigated the impact of aortic prosthesis size, and thus PPM, on the evolution of FMR. Our findings revealed that patients without PPM experienced not only significant reductions in postoperative peak and mean gradients but also beneficial

reverse remodeling of the LV, as evidenced by reductions in LVEDD, LVESD, septum thickness, and LV posterior wall thickness. Furthermore, the improvement in FMR grade below 2+ was notably higher in the non-PPM group (68.6%) compared to the PPM group (59.2%). It is important to note that our study employed an identical model of mechanical and tissue prostheses across all patients, thereby eliminating the potential influence of different manufacturer designs on our results. However, contrasting findings by Waisbren et al. suggested no independent relationship between aortic prosthesis size and changes in MR [26].

Limitations

While our study benefited from a well-defined and homogeneous FMR group due to restrictive patient selection criteria, the limitation of a small sample size, particularly in comparing the non-PPM and PPM groups (86 vs. 22 patients, respectively), must be acknowledged. Calculating the EOAi based on the manufacturer's published values, rather than on patient-specific post-surgical measurements, may limit the precision of our findings. This approach was chosen due to limited availability of direct postoperative measurements. The accuracy of EOA echocardiographic measurement in bioprosthetic valves is restricted by similar challenges to those in AVA measurement; the LV outflow tract diameter could be difficult to measure due to reverberation artifacts from the prosthetic valve. For bileaflet mechanical valves, the central orifice could produce a high-velocity jet, leading to potential EOA underestimation. We recognize the possibility that these projected values may overestimate EOAi and the incidence of PPM. Larger studies with more robust patient numbers are needed to validate the relationships between aortic prosthesis size, PPM, and the evolution of FMR following AVR.

5. Conclusions

Our study showed an improvement in FMR following AVR surgery in the majority of patients. Our findings also suggest that PPM may adversely affect the reduction of FMR. Optimal prosthesis sizing and selection are crucial for maximizing the therapeutic benefits of AVR, including the mitigation of secondary MR. We advocate selecting a prosthesis of adequate size to optimize hemodynamic outcomes and mitigate the risk of residual or worsening FMR postoperatively. We support a tailored surgical approach that prioritizes optimizing prosthesis size to avoid PPM, thereby potentially improving outcomes and reducing the need for additional MV interventions in this patient population.

Author Contributions: Conceptualization, P.D., S.B. and J.P. Formal analysis, D.A.; investigation, P.D., S.B. and B.V.; methodology, P.D. and S.B.; resources, M.S.; software, D.A.; validation, S.P., M.N. and J.P. Writing—original draft, P.D., M.S. and S.P.; writing—review and editing, D.A., M.N., S.B. and J.P. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Ethical review and approval were waived for this study due to data being anonymous, and the study not involving any additional patient intervention or access to identifiable personal information, since data were collected for standard clinical purposes.

Informed Consent Statement: Individual consent was waived due to the nature of the data being fully anonymized and retrospective in nature, meaning that no personally identifiable information could be traced back to specific individuals. Given that the data was collected for standard clinical purposes and was irreversibly anonymized prior to analysis, obtaining individual consent was not deemed necessary according to institutional guidelines and relevant ethical standards.

Data Availability Statement: The data presented in this study are available on request from the corresponding author due to ethical reasons.

Conflicts of Interest: The authors declare no conflicts of interest.

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Progression of Non-Significant Mitral and Tricuspid Regurgitation after Surgical Aortic Valve Replacement for Aortic Regurgitation

Shirit Kazum [1,2], Mordehay Vaturi [1,2], Idit Yedidya [1,2], Shmuel Schwartzenberg [1,2], Olga Morelli [1,2], Keren Skalsky [1,2], Hadas Ofek [1,2], Ram Sharony [2,3], Ran Kornowski [1,2], Yaron Shapira [1,2] and Alon Shechter [1,2,4,*]

[1] Department of Cardiology, Rabin Medical Center, Petach Tikva 4941492, Israel
[2] Faculty of Medicine, Tel Aviv University, Tel Aviv 6997801, Israel
[3] Department of Thoracic Heart Surgery, Rabin Medical Center, Petach Tikva 4941492, Israel
[4] Department of Cardiology, Smidt Heart Institute, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA
* Correspondence: alonshechter@gmail.com; Tel.: +1-(310)-423-2726; Fax: +1-(310)-423-0166

Abstract: Little is known about the natural history of non-significant mitral and tricuspid regurgitation (MR and TR) following surgical aortic valve replacement (SAVR) for aortic regurgitation (AR). We retrospectively analyzed 184 patients (median age 64 (IQR, 55–74) years, 76.6% males) who underwent SAVR for AR. Subjects with significant non-aortic valvulopathies, prior/concomitant valvular interventions, or congenital heart disease were excluded. The cohort was evaluated for MR/TR progression and, based on the latter's occurrence, for echocardiographic and clinical indices of heart failure and mortality. By 5.8 (IQR, 2.8–11.0) years post-intervention, moderate or severe MR occurred in 20 (10.9%) patients, moderate or severe TR in 25 (13.5%), and either of the two in 36 (19.6%). Patients who developed moderate or severe MR/TR displayed greater biventricular disfunction and functional limitation and were less likely to be alive at 7.0 (IQR, 3.4–12.1) years compared to those who did not (47.2 vs. 79.7%, $p < 0.001$). The emergence of significant MR/TR was associated with preoperative atrial fibrillation/flutter, symptomatic heart failure, and above-mild MR/TR as well as concomitant composite graft use, but not with baseline echocardiographic measures of biventricular function and dimensions, aortic valve morphology, or procedural aspects. In conclusion, among patients undergoing SAVR for AR, significant MR/TR developed in one fifth by six years, correlated with more adverse course, and was anticipated by baseline clinical and echocardiographic variables.

Keywords: mitral regurgitation; tricuspid regurgitation; surgical aortic valve replacement; aortic regurgitation; progression

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1. Introduction

Aortic regurgitation (AR) is a condition in which the aortic valve (AV) fails to prevent systemic blood from back flowing into left ventricle (LV) during diastole. Constituting one of the most common valvular diseases in adults worldwide, AR usually manifests as a slowly progressive disease characterized by a gradual increase in LV volume load, a compensatory rise in chamber size and mass (i.e., eccentric remodeling and hypertrophy), and finally biventricular malfunction—ultimately translating to clinical heart failure (HF). At present, the definite treatment of unrepairable significant AR accompanied by signs or symptoms of cardiac dysfunction is surgical AV replacement (SAVR). While possible, addressing additional valvulopathies at the time of operation comes at the price of a lengthier procedure, potential complications, and higher costs, all of which could outweigh any theoretical benefit of a one-time multivalvular intervention. Yet, considering the worse prognosis and increased mortality associated with the co-presence of significant AR and mitral and/or tricuspid regurgitation (MR and/or TR) [1–3], current position papers and

practice guidelines advocate the latter's correction in parallel to SAVR [4–6]. Still, there is no consensus regarding the management of non-significant (i.e., less than moderate) MR and/or TR at the time of SAVR for AR, reflecting the paucity of data on the natural history of these valvular disorders in the context of AR, as most studies to date have focused on patients with either stenotic or mixed (rather than regurgitant) AV pathologies [7–11]. As a first step towards improving the decision-making process in this area of uncertainty, we examined the frequency of less than moderate MR or TR deterioration following SAVR for AR and further evaluated predictors for its occurrence, all using a large and contemporary database.

2. Materials and Methods

2.1. Study Population and Outcomes

Our study is based on the Rabin Medical Center registry of consecutive SAVR procedures performed for moderate-to-severe or severe AR on adult patients between 1 January 1996 and 31 December 2020. Included in the study were patients who exhibited less than moderate MR or TR at the baseline and for whom there was at least one retrievable transthoracic echocardiogram (TTE) prior to SAVR and two after it, one of them within the first six postprocedural months. We excluded patients with any of the following: 1. Greater than mild mitral or tricuspid stenosis; 2. Prior or concomitant non-aortic valvular interventions; 3. Concurrent LV assist device implantation; 4. Congenital heart disease; and 5. Acute intra- or postprocedural development of significant MR or TR due to a surgical complication.

The primary outcome was the incidence of MR or TR progression to moderate or severe on the last documented TTE. Based on the occurrence of this composite endpoint, the cohort was also retrospectively assessed for accompanying echocardiographic indices of ventricular and valvular function, New York Heart Association (NYHA) functional class at 1-year and at the last visit, and all-cause mortality along the entire follow-up period.

Conforming to the Declaration of Helsinki, the study was approved by Rabin's Institutional Review Board (number 0603-23-RMC) which waived the need for informed consent.

2.2. Procedural Aspects

SAVR was undertaken following a dedicated heart team discussion that considered the best medical evidence at the time, practice guidelines [5,6], and patient preferences. Most procedures were performed via median sternotomy. Cardiopulmonary bypass was achieved by ascending aortic and double-stage venous cannulations, utilizing antegrade moderate hypothermic (28–30 degrees Celsius) cardioplegia. Actual valve replacement was performed according to standard pledged and interrupted-suture techniques. Transesophageal echocardiography and right heart catheterization were used for guidance, monitoring, and evaluation of the surgical result, as appropriate.

2.3. Echocardiographic Assessment

Echocardiograms at all stages were performed and interpreted by a team of experienced sonographers and level III-trained echocardiologists in accordance with accepted guidelines [12–15]. The echo machines used were Sonos-5500, Sonos-7500, IE-33, and EPIQ-7 (Philips, Andover, MA, USA) as well as Vivid-7 and Vivid-I (General Electric, Boston, MA, USA).

Regurgitation severity at all positions was determined in real-time by integration of qualitative (e.g., color Doppler-driven) and (semi)quantitative (e.g., spectral Doppler-derived) measures, whenever feasible, and graded as 0 (none-to-minimal), 1 (mild or mild-to-moderate), 2 (moderate), 3 (moderate-to-severe), or 4 (severe and greater). For the purpose of the study and in view of the guidelines, MR and/or TR of moderate, moderate-to-severe, and severe degrees were collectively referred to as "moderate or severe." In cases of diagnostic ambiguity regarding AR extent, a multimodality approach was employed as deemed appropriate by the treating team, which utilized cardiac magnetic resonance

and/or cardiac computed tomography for better volumetric assessment of regurgitant fraction and LV function and dimensions [16,17].

Global right ventricular (RV) function was assessed qualitatively and RV dilatation was defined as an end-diastolic RV diameter of 4.2 cm or greater by the apical 4-chamber view.

2.4. Data Collection

Echocardiographic parameters were retrieved from electronically stored reports, which were verified and amended as needed by a consensus of at least two echocardiologists taking part in the heart team meetings. Clinical data, including past medical history, medications, procedures, providers' notes, and test results, were extracted from a web-based medical chart (Ofek, dbMotion, Pittsburg, PA, USA) shared by all Israeli hospitals and health maintenance organizations. Demographic and mortality details were verified using governmental registries.

2.5. Statistical Analysis

The study cohort was analyzed in its entirety and based on the occurrence of the primary outcome. Variables were reported as frequencies and percentages, medians and interquartile ranges (IQRs), or means and standard deviations. Inter-group differences were evaluated using Pearson's chi-square, Fisher's exact, Mann–Whitney U, or Student's *t* tests, as suitable. Change over time in the NYHA class was assessed by the McNemar test.

To identify potential predictors for the primary outcome, a multivariable binary logistic regression analysis was constructed which incorporated baseline and procedural variables of perceived prognostic value that also possessed a *p*-value of <0.1 on univariable models.

A two-sided *p*-value of <0.05 defined statistical significance. Cases with missing data were censored from the relevant calculations. All analyses were performed using SPSS, version 24 (IBM Corporation, Armonk, NY, USA).

3. Results

3.1. Baseline Characteristics of the Study Cohort

A total of 184 patients entered the analysis and were followed for 7.0 (IQR, 3.4–12.1) years (Figure 1). The study cohort had a median age of 64 (IQR, 55–74) years and a male predominance (n = 141, 76.6%) (Table 1). A little more than half (n = 96, 53.0%) of patients presented to surgery with symptomatic HF (i.e., NYHA class II and above).

AR was mainly isolated (n = 134, 72.8%) and the leading AR etiology was annular dilatation (Table 2). Bicuspid AV and significant (i.e., a $\geq$ 4.5-cm) ascending aortic dilatation were each observed in approximately a third of cases (n = 60, 34.1% and n = 50, 29.8%, respectively). Mild-to-moderate MR or TR affected at baseline 47 (25.5%) patients, 41 (87.2%) of whom displayed only MR. The most common mitral structural anomaly was prolapse and/or flail (n = 44, 23.9%), followed by rheumatic disease (n = 14, 7.6%) and annular calcification (n = 8, 4.3%).

3.2. Procedural Aspects

Most surgeries were elective and non-urgent and involved biologic valve implantation (Table 3). Overall, 25% (n = 46) incorporated an ascending aortic and/or aortic root replacement and close to one fifth (n = 32, 17.5%) were accompanied by coronary artery bypass grafting.

3.3. Outcomes

The last echocardiogram, performed at 5.8 (IQR, 2.8–11.0) years after surgery, revealed the primary outcome, namely a composite of moderate or severe MR or TR, in 36 (19.6%) patients (Table 4). Concomitantly, moderate or severe MR developed in 20 (10.9%) cases, moderate or severe TR in 25 (13.6%), and both in 9 (4.9%). New-onset severe MR or TR occurred in 26 (14.1%) patients.

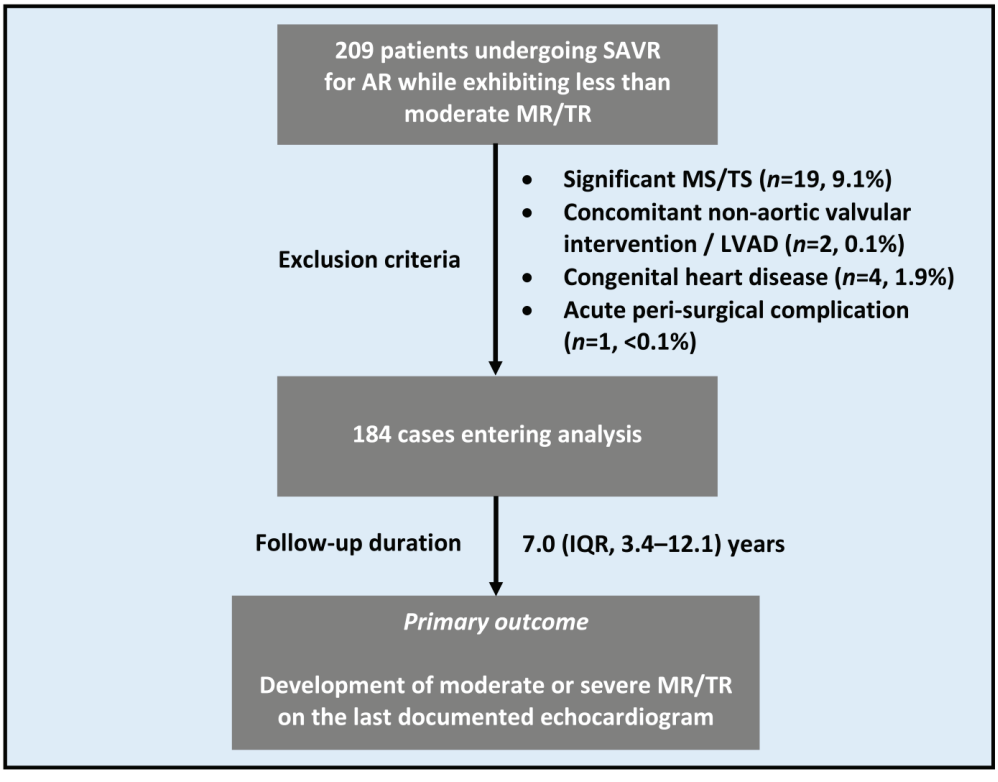


Figure 1. Study Flow Chart. AR = aortic regurgitation; IQR = interquartile range; LVAD = left ventricular assist device; MR = mitral regurgitation; MS = mitral stenosis; SAVR = surgical aortic valve replacement; TR = tricuspid regurgitation; TS = tricuspid stenosis.

Resembling the preprocedural stage, the primary outcome group experienced greater functional incapacitation at one year and at the last follow-up visits (Figure 2), the latter of which proved more profound compared to the baseline ($p = 0.044$), as opposed to the non-significant difference between the baseline and last NYHA status within the no primary outcome group ($p = 0.205$). All-cause mortality rate along the entire follow-up period was also higher among patients who developed moderate or severe MR or TR ($n = 19$, 52.8% vs. $n = 30$, 20.3%, $p < 0.001$) and the risk for mortality was increased by the emergence of moderate or severe MR or TR according to univariate analysis (HR 1.78, 95% CI 1.10–3.18, $p = 0.035$). While death causes were mainly non-cardiovascular and equally distributed in the two study groups, mortality among patients sustaining the primary outcome tended to be cardiovascular more often ($n = 8/19$, 42.1% vs. $n = 10/30$, 33.3%, $p = 0.081$) (Supplemental Table S1).

3.4. Correlates of the Primary Outcome

Compared to patients who did not display moderate or severe MR or TR, those who did were more likely, at baseline, to exhibit atrial fibrillation/flutter, symptomatic HF, LV dysfunction, mitral valve structural abnormalities, and mild-to-moderate (vs mild or less) MR and/or TR. Also, they had a non-significantly larger ascending aortic diameter (but a marginally lower prevalence of bicuspid AV) and underwent composite graft implantation at the time of surgery more frequently. Notably, a higher incidence of moderate or severe TR, as well as of moderate or severe MR or TR, was observed among patients with mild-to-moderate (vs up-to-mild) TR or MR/TR prior to SAVR (Supplemental Table S2). The

development of moderate or severe MR alone was independent of baseline MR, TR, and MR/TR severity.

Table 1. Baseline Clinical Characteristics.

	Total Cohort (*n* = 184)	Primary Outcome (*n* = 36)	No Primary Outcome (*n* = 148)	*p*-Value
Demographic Data				
Age				
Median (years)	64 (55–74)	70 (57–76)	62 (54–73)	0.112
≥65 years	89 (48.4)	21 (58.3)	68 (45.9)	0.182
Sex Male	141 (76.6)	24 (66.7)	117 (79.1)	0.115
Comorbidities				
Body Surface Area, Mosteller Formula (m^2)	1.9 (1.8–2.1)	1.9 (1.7–2.0)	1.9 (1.8–2.1)	0.207
Body Mass Index (kg/m^2)	27.8 (24.5–30.3)	28.1 (24.0–29.3)	27.6 (24.6–31.1)	0.845
Obesity	57 (33.9)	8 (24.2)	49 (36.3)	0.190
Hypertension	122 (70.1)	25 (69.4)	97 (70.3)	0.921
Diabetes Mellitus	53 (30.6)	11 (30.6)	42 (30.7)	0.991
Dyslipidemia	135 (78.0)	29 (80.6)	106 (77.4)	0.681
Smoking History	35 (20.2)	5 (13.9)	30 (21.9)	0.287
Estimated Glomerular Filtration Rate, Cockcroft Formula (mL/kg/min)	86.7 (67.9–113.5)	78.6 (60.9–112.3)	89.1 (70.1–114.2)	0.298
Stage ≥ III Chronic Kidney Disease	29 (18.8)	7 (23.3)	22 (17.7)	0.482
Ischemic Heart Disease	64 (36.8)	18 (50.0)	46 (33.3)	0.065
Prior Stroke/Transient Ischemic Attack	23 (14.6)	8 (25.0)	15 (11.9)	0.088
Atrial Fibrillation/Flutter	65 (38.2)	22 (61.1)	43 (32.1)	0.001
Cardiac Implantable Electronic Device	20 (11.8)	6 (16.7)	14 (10.5)	0.381
Marfan Syndrome	1 (0.6)	0 (0.0)	1 (0.7)	1.000
Symptomatic Status				
New York Heart Association Class				0.012
I	85 (47.0)	9 (25.0)	76 (52.4)	
II	73 (40.3)	21 (58.3)	52 (35.9)	
III	23 (12.7)	6 (16.7)	17 (11.7)	
≥II	96 (53.0)	27 (75.0)	69 (47.6)	0.005

Data are presented as number (percent) or median (interquartile range).

Table 2. Baseline Echocardiographic Parameters.

	Total Cohort (*n* = 184)	Primary Outcome (*n* = 36)	No Primary Outcome (*n* = 148)	*p*-Value
Study Time Prior to Surgery (days)	52 (13–192)	51 (18–182)	48 (11–208)	0.563
Aortic Valve				
Pure Aortic Regurgitation	134 (72.8)	30 (83.3)	104 (70.3)	0.114
Aortic Regurgitation Severity				0.511
Moderate-to-Severe	101 (54.9)	18 (50.0)	83 (56.1)	
Severe	83 (45.1)	18 (50.0)	65 (43.9)	
Aortic Regurgitation Etiology				0.421
Annular Dilatation	79 (65.8)	16 (64.0)	63 (66.3)	
Leaflet Prolapse/Flail	14 (11.7)	4 (16.0)	10 (10.5)	
Leaflet Restriction	10 (8.3)	3 (12.0)	7 (7.4)	
Endocarditis	15 (12.5)	1 (4.0)	14 (14.7)	
Aortic Dissection	2 (1.7)	1 (4.0)	1 (1.1)	
Moderate and Above Aortic Stenosis	54 (29.3)	6 (16.7)	48 (32.4)	0.062
Bicuspid Aortic Valve	60 (34.1)	7 (20.0)	53 (37.6)	0.049

Table 2. *Cont.*

	Total Cohort (*n* = 184)	Primary Outcome (*n* = 36)	No Primary Outcome (*n* = 148)	*p*-Value
Aorta				
Aortic Root Diameter (cm)	3.6 (3.0–4.1)	3.5 (2.9–4.3)	3.6 (3.0–4.1)	0.754
Ascending Aortic Diameter				
Median (cm)	4.1 (3.6–4.7)	4.2 (3.9–4.9)	4.0 (3.5–4.6)	0.065
≥4 cm	96 (57.1)	21 (70.0)	75 (54.3)	0.116
≥4.5 cm	50 (29.8)	11 (36.7)	39 (28.3)	0.361
Mitral and Tricuspid Valves				
Mitral Valve Anomalies				
Rheumatic Changes	14 (7.6)	3 (8.3)	11 (7.4)	0.739
Annular Dilatation	1 (0.5)	0 (0.0)	1 (0.7)	0.621
Annular Calcification	8 (4.3)	6 (16.7)	2 (1.4)	0.001
Leaflet Prolapse/Flail	44 (32.8)	13 (43.3)	31 (29.8)	0.165
Leaflet Restriction	8 (4.3)	4 (11.1)	4 (2.7)	0.048
Leaflet Tethering/Retraction	5 (2.7)	4 (11.1)	1 (0.7)	0.005
Diastolic Mitral Regurgitation	1 (0.5)	0 (0.0)	1 (0.7)	0.621
Mitral and Tricuspid Regurgitation Grade				
Mitral	0.7 ± 0.5	0.9 ± 0.3	0.6 ± 0.5	<0.001
Tricuspid	0.4 ± 0.5	0.5 ± 0.5	0.4 ± 0.5	0.164
Mild-to-Moderate Mitral or Tricuspid Regurgitation				
Mitral	41 (22.3)	15 (41.7)	26 (17.6)	0.002
Tricuspid	14 (7.7)	6 (17.1)	8 (5.4)	0.030
Either	47 (25.5)	16 (44.4)	31 (20.9)	0.004
Left Heart Chambers				
Left Ventricular Ejection Fraction				
Median (%)	60 (45–60)	50 (41–60)	60 (50–60)	0.005
<50%	52 (28.9)	16 (44.4)	36 (25.0)	0.021
Regional Wall Motion Abnormality	19 (10.3)	5 (13.9)	14 (9.5)	0.540
Left Ventricular Diastolic Dysfunction				
Any	66 (75.9)	7 (87.5)	59 (74.7)	0.673
Grade ≥2	14 (16.1)	1 (12.5)	13 (16.5)	0.772
Left Ventricular End-Systolic Diameter (cm)	3.8 (3.3–4.5)	4.1 (3.4–4.9)	3.8 (3.3–4.4)	0.213
Left Ventricular End-Diastolic Diameter (cm)	5.7 (5.2–6.3)	6.1 (5.1–6.4)	5.7 (5.2–6.1)	0.210
Left Atrial Diameter (cm)	4.2 (3.8–4.6)	4.4 (3.9–4.7)	4.1 (3.8–4.6)	0.361
Left Atrial Area (cm^2)	23.8 (20.0–27.0)	25.5 (21.5–29.0)	23.0 (19.5–26.5)	0.072
Right Heart Chambers				
Right Ventricular Dysfunction	8 (4.5)	3 (8.8)	5 (3.5)	0.184
Right Ventricular Dilatation	3 (1.7)	1 (2.9)	2 (1.4)	0.477
Tricuspid Annular Systolic Plane Excursion (mm)	22.5 (16.8–26.8)	15.0 (13.0–16.0)	23.0 (19.0–27.0)	0.250
Pulmonary Arterial Systolic Pressure				
Median (mmHg)	27 (21–35)	32 (22–39)	26 (21–33)	0.103
>40 mmHg	8 (5.7)	4 (12.9)	4 (3.6)	0.070

Data are presented as number (percent), median (interquartile range), or mean ± standard deviation.

Following SAVR, the primary outcome group exhibited a nominally higher residual AR grade, worse biventricular function, more pronounced chamber dilatation, and higher pulmonary arterial systolic pressure on the last documented echocardiogram (Table 4).

3.5. Predictors of the Primary Outcome

After multivariable analysis, four parameters were identified that independently conferred a higher risk for the emergence of moderate or severe MR or TR: the presence of atrial fibrillation/flutter (OR 3.30, 95% CI 1.10–9.85, $p = 0.033$), symptomatic HF (OR 7.42, 95% CI 3.47–14.82, $p = 0.004$), and mild-to-moderate (vs up-to-mild) MR or TR (OR 4.17,

95% CI 1.35–12.91, $p = 0.013$) preprocedure, and the use of composite graft during surgery (OR 4.20, 95% CI 1.29–13.61, $p = 0.017$) (Table 5 and Supplemental Table S3). Risk factor distribution and the probability of this composite endpoint as a function of the number of risk factors are presented in Figure 3. Interestingly, neither echocardiographic measures of chamber function and dimensions, nor aortic/mitral valve morphology or surgical aspects, were predictive of the primary outcome.

Table 3. Procedural Aspects.

	Total Cohort ($n = 184$)	Primary Outcome ($n = 36$)	No Primary Outcome ($n = 148$)	p-Value
Urgent Surgery	9 (4.9)	2 (5.6)	7 (4.8)	0.850
Aortic Valve Prosthesis Type				0.295
Biologic	130 (70.7)	28 (77.8)	102 (68.9)	
Mechanical	54 (29.3)	8 (22.2)	46 (31.1)	
Concomitant Aortic Vascular Intervention				
Any	46 (25.0)	11 (30.6)	35 (23.6)	0.391
Composite Graft Implantation	35 (21.6)	11 (37.9)	24 (18.0)	0.018
Concomitant Coronary Artery Bypass Grafting	32 (17.5)	8 (22.2)	24 (16.3)	0.404

Data are presented as number (percent) or median (interquartile range).

Table 4. Last Echocardiographic Findings.

	Total Cohort ($n = 184$)	Primary Outcome ($n = 36$)	No Primary Outcome ($n = 148$)	p-Value
Study Time After Surgery (years)	5.8 (2.8–11.0)	5.6 (2.7–10.8)	5.8 (2.9–11.1)	0.724
Aortic Valve				
Residual Aortic Regurgitation Severity				
Up-to-Mild	180 (97.8)	34 (94.4)	146 (98.6)	0.172
Moderate	3 (1.6)	2 (5.6)	1 (0.7)	0.098
Above-Moderate	1 (0.5)	0 (0.0)	1 (0.7)	1.000
Residual Aortic Regurgitation Grade	0.2 ± 0.5	0.4 ± 0.6	0.2 ± 0.5	0.081
Moderate and Above Aortic Stenosis	1 (0.5)	0 (0.0)	1 (0.7)	1.000
Mitral and Tricuspid Valves				
Moderate or Severe Mitral or Tricuspid Regurgitation				<0.001
Mitral	20 (10.9)	20 (55.6)	0 (0.0)	
Tricuspid	25 (13.6)	25 (69.4)	0 (0.0)	
Either	36 (19.7)	36 (100.0)	0 (0.0)	
Both	9 (4.9)	9 (25.0)	0 (0.0)	
Severe Mitral or Tricuspid Regurgitation	26 (14.1)	26 (72.2)	0 (0.0)	<0.001
Mitral Regurgitation Grade				
Median	0.8 ± 0.9	$2.0 (\pm 1.3)$	0.5 ± 0.5	<0.001
Change from Baseline	0.2 ± 0.9	1.1 ± 1.2	-0.1 ± 0.6	<0.001
Tricuspid Regurgitation Grade				
Median	0.8 ± 1.0	2.3 ± 1.3	0.5 ± 0.5	<0.001
Change from Baseline	0.4 ± 1.0	1.7 ± 1.1	0.1 ± 0.6	<0.001

Table 4. *Cont.*

	Total Cohort (*n* = 184)	Primary Outcome (*n* = 36)	No Primary Outcome (*n* = 148)	*p*-Value
Left Heart Chambers				
Left Ventricular Ejection Fraction				
Median (%)	60 (50–60)	55 (31–60)	60 (50–60)	0.010
Change from Baseline (%)				
Absolute	0 (−5–5)	0 (−12–5)	0 (−4–5)	0.172
Relative	0.0 (−8.3–10.0)	0.0 (−25.0–11.9)	0.0 (−7.5–10.0)	0.150
<50%	41 (22.8)	16 (44.4)	25 (17.4)	0.001
Left Ventricular End-Systolic Diameter				
Median (cm)	3.1 (2.7–3.7)	3.4 (2.8–4.9)	3.0 (2.7–3.6)	0.011
Change from Baseline				
Absolute (cm)	−0.7 (−1.3–0.0)	−0.5 (−1.3–1.6)	−0.7 (−1.3–[−0.1])	0.097
Relative (%)	−18.7 (−30.3–0.0)	−12.5 (−31.3–12.7)	−19.4 (−30.2–[−2.7])	0.163
Left Atrial Diameter				
Median (cm)	4.3 (3.7–4.9)	5.0 (4.5–5.4)	4.2 (3.7–4.7)	<0.001
Change from Baseline				
Absolute (cm)	0.1 (−0.4–0.8)	0.8 (−0.2–1.3)	0.1 (−0.5–0.7)	0.010
Relative (%)	2.8 (−10.1–19.4)	18.6 (−3.7–30.2)	2.1 (−11.1–17.0)	0.013
Right Heart Chambers				
Right Ventricular Dysfunction	20 (11.8)	13 (40.6)	7 (5.1)	<0.001
Right Ventricular Dilatation	22 (12.9)	10 (30.3)	12 (8.7)	0.001
Pulmonary Arterial Systolic Pressure				
Median (mmHg)	29 (22–34)	35 (30–46)	26 (22–32)	<0.001
Change from Baseline				
Absolute (mmHg)	0 (−8–8)	4 (−6–18)	−1 (−9–6)	0.117
Relative (%)	−4.3 (−26.6–26.8)	10.5 (−17.2–63.6)	−8.0 (−27.3–20.3)	0.063
>40 mmHg	17 (12.7)	10 (31.3)	7 (6.9)	0.001

Data are presented as number (percent), median (interquartile range), or mean ± standard deviation.

Table 5. Multivariable Binary Logistic Regression Model for the Primary Outcome.

	OR (95% CI)	*p*-Value
Age (Continuous)	0.99 (0.94–1.04)	0.599
Ischemic Heart Disease	1.22 (0.41–3.61)	0.724
Prior Stroke/Transient Ischemic Attack	2.39 (0.66–8.58)	0.182
Atrial Fibrillation/Flutter	3.30 (1.10–9.85)	0.033
New York Heart Association Class ≥ II	7.42 (3.47–14.82)	0.004
Bicuspid Aortic Valve	0.37 (0.09–1.50)	0.163
Mild-to-Moderate Mitral or Tricuspid Regurgitation	4.17 (1.35–12.91)	0.013
Left Ventricular Ejection Fraction (continuous)	0.98 (0.93–1.03)	0.446
Composite Graft Use	4.20 (1.29–13.61)	0.017

CI = confidence interval; OR = odds ratio.

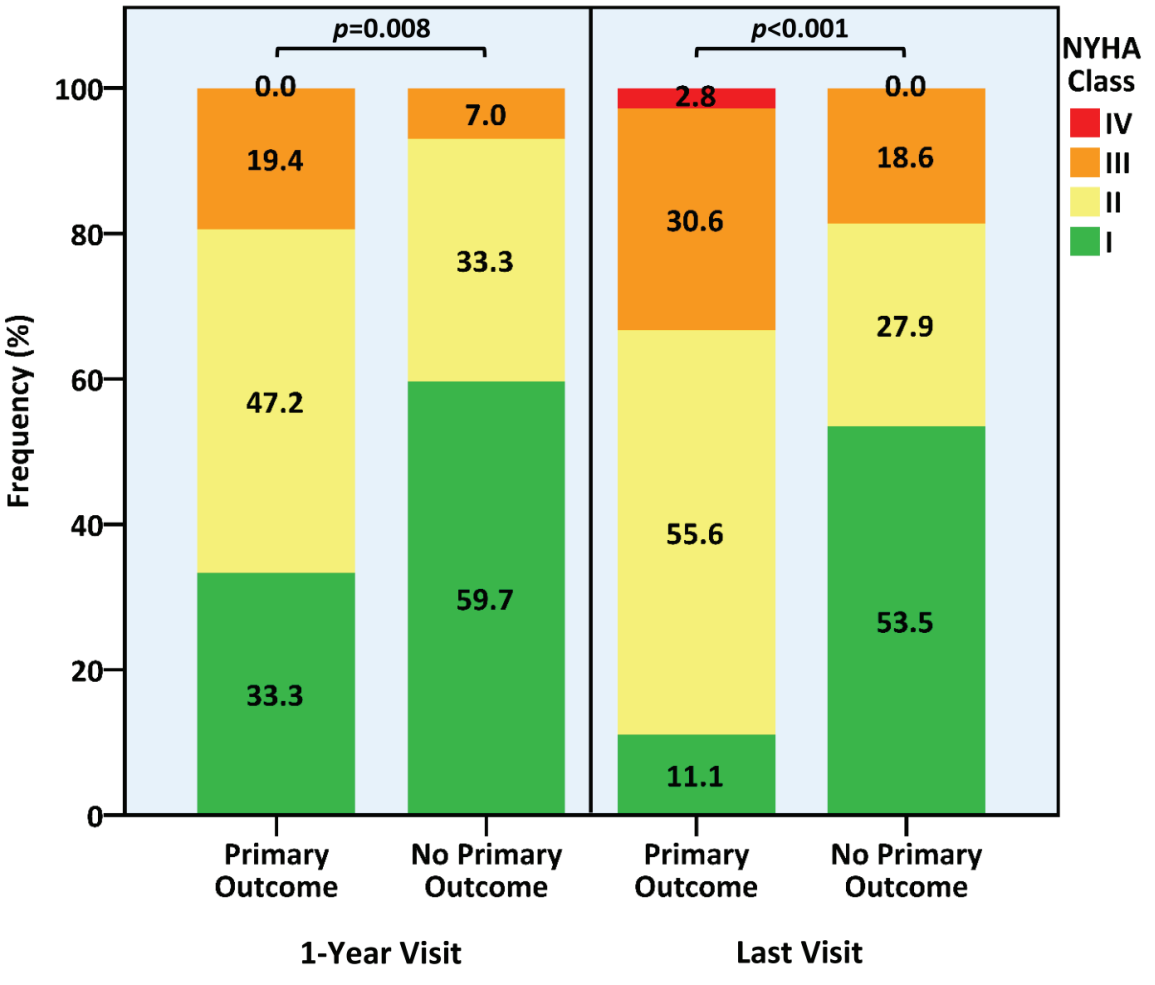
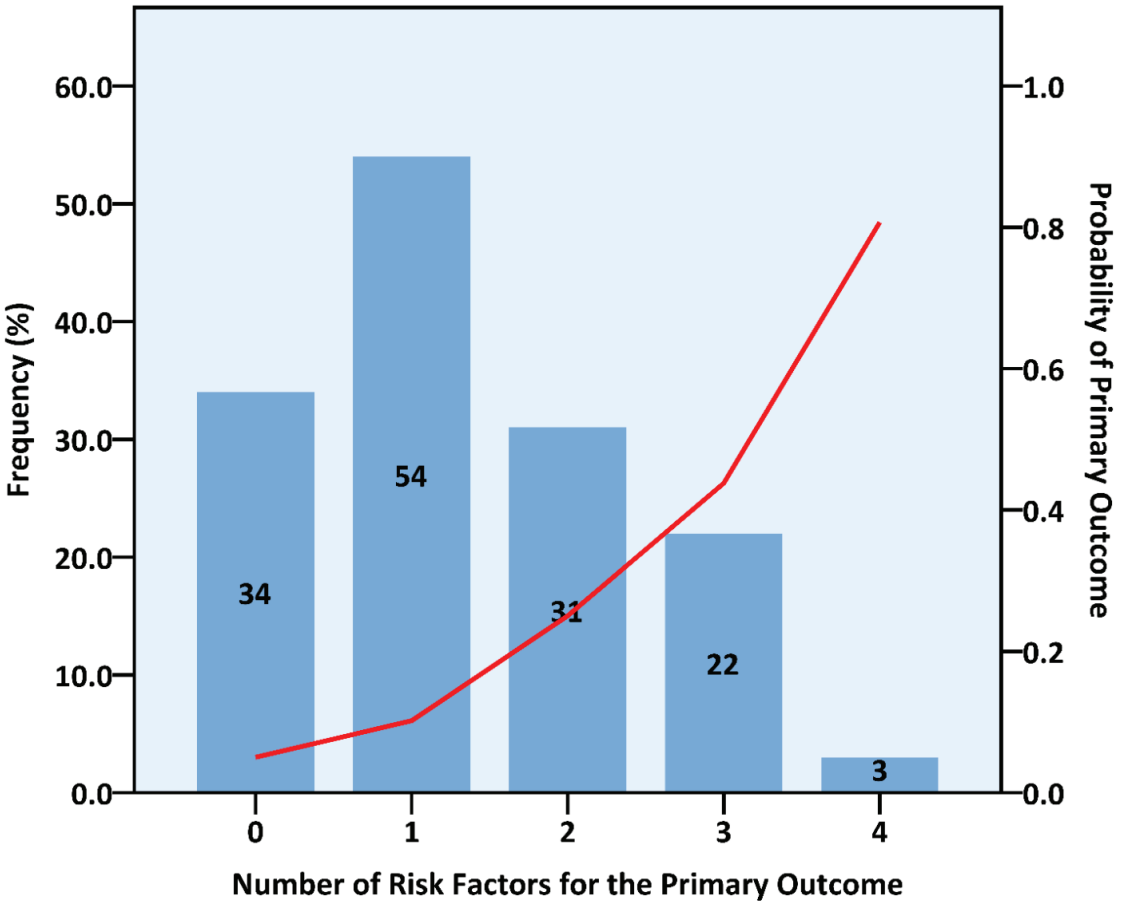


Figure 2. Post-Procedural Functional Status According to the Occurrence of the Primary Outcome. NYHA = New York Heart Association.



Risk Factor	OR (95% CI)	*p*-Value
Atrial Fibrillation/Flutter	3.30 (1.10–9.85)	0.033
New York Heart Association Class ≥II	7.42 (3.47–14.82)	0.004
Mild-to-Moderate Mitral or Tricuspid Regurgitation	4.17 (1.35–12.91)	0.013
Composite Graft Use	4.20 (1.29–13.61)	0.017

Figure 3. Risk Factor Burden Distribution and Correlation with the Primary Outcome. Bars represent the prevalence, at baseline, of the various risk factors counts. Red line illustrates the forecasted odds ratio for the occurrence of the primary outcome that was associated with each observed number of preprocedural risk factors. CI = confidence interval; OR = odds ratio.

4. Discussion

Our study evaluated the long-term progression of non-significant MR and TR following SAVR for AR. Analyzing the data of a single-center, 184-patient cohort, the great majority (72.8%) of which displayed pure AR, we found that: 1. The primary composite outcome of moderate or severe MR or TR development occurred in about one in five cases within six years after the intervention; 2. Patients with new-onset moderate or severe MR or TR tended to exhibit a greater residual AR and were more likely to suffer biventricular dysfunction and dilatation and pulmonary hypertension on the last documented

echocardiogram; 3. The emergence of moderate or severe MR or TR was associated with worse functional status and increased all-cause mortality rate during the study's seven-year follow-up period; and 4. The risk for the development of moderate or severe MR or TR was higher in the presence of preprocedural atrial fibrillation/flutter, symptomatic HF, and above-mild MR or TR, as well as by implantation of composite graft during the index operation.

Current data regarding the course of MR and TR after AV replacement (AVR) are derived from studies that either focused on aortic stenosis (AS) or highly-selected AR cases or utilized a rather short follow-up duration. Among patients with AS, MR grade has been shown to improve overtime following both surgical [18] and transcatheter [19] AVR, while TR worsening has been observed in up to 17% of cases post-procedure, inflicting lower survival [20–23]. In patients with AR, mild MR has deteriorated in 4% of patients after SAVR according to one report [24] and moderate or severe MR has occurred in 9.4% according to another [25]. Notably, the former study spanned 3.2 years of follow-up and found a direct correlation between the follow-up time and MR progression, whereas the latter, representing 10 ± 4 years of follow-up, analyzed 97 patients, all with bicuspid AV. Our study, with its novel design, longer surveillance time, and less strict inclusion criteria therefore provides robust and real-world data on the deterioration of MR or TR following SAVR for AR, which, according to our findings, could be relevant to a non-negligible portion of patients.

Three notions may be stressed based on the study's results. The first is that MR or TR progression post SAVR for AR is a common phenomenon associated with more advanced HF and reduced survival. Although the last documented residual AR was non-significant (i.e., up-to-moderate) in most patients, the overall AR grade (as a continuous variable) was nominally higher among those experiencing the primary outcome, suggesting a potential link between the simultaneous deterioration of the three regurgitant lesions. Whether worsening of one valvular insufficiency mediated the other or whether all the three simply represented a common underlying pathology (e.g., cardiomyopathy, connective tissue disease, or inflammatory disorder) that was not addressed by the mere AV operation, is an interesting question that could not be reliably answered by our retrospective and small-scale analysis. As for the reason accounting for the increased functional incapacitation and mortality observed among patients with MR or TR deterioration post-SAVR, our findings suggest a cardiovascular-originated mechanism. This is in view of the numerically higher cardiovascular death rate as well as the more pronounced myocardial derangement (reflected by worse ventricular function and dilatation) that accompanied MR or TR progression. Once again, and considering the study's design, we could not determine causality, stressing the need for larger, prospective research.

The second notion arising from our work is that the development of significant MR or TR after SAVR for AR could be anticipated based on easily measurable conditions prior to the intervention. Regarding atrial fibrillation/flutter, it could be that the arrhythmic aberration partially counteracted the beneficial effect of AR correction on cardiopulmonary hemodynamics and myocardial remodeling [26,27]. Symptomatic HF and pre-existent MR or TR, on their part, might have also expressed a more profound disease state initially, as suggested by the lower LVEF observed among patients who sustained moderate or severe MR or TR post-procedure. The mechanism responsible for the association between concomitant replacement of the aorta and MR or TR progression may have been related to a more widespread disease at the outset as well or again to the presence of a shared pathology such as collagen/elastin disorders. For this matter, while aortic root and ascending aortic diameters were not independently predictive of the risk for the primary outcome per se, a nominally larger ascending aortic diameter was nevertheless noted among patients who developed moderate or severe MR or TR. Considering similarities in body habitus, general comorbidities, and immediate AR etiologies across the two study groups, this finding could imply the existence of an intrinsic under-diagnosed connective tissue-related condition(s). Importantly, pre-operative morphological (e.g., rheumatic, calcific, or degenerative) aber-

rations at the mitral position, although not significantly associated with the risk for the occurrence of moderate or severe MR or TR, were also more common in patients who exhibited the primary outcome, thus suggesting a role for baseline structural anomalies in MR or TR progression too, as well as supporting the possibility of an underlying common disease process. As for parameters not shown to correlate with the risk for the primary outcome, it is plausible that the study's small sample size and low number of observations and events prevented the appreciation of additional relevant predictors, mainly specific regurgitation etiologies (rheumatic heart disease in particular [22]), RV dysfunction, and pulmonary hypertension. These may be evaluated by future larger explorations as well.

On a final and more practical note, our study underscores the importance of guideline-directed multi-modality evaluation and management of those preprocedural conditions shown to be associated with MR or TR deterioration post SAVR for AR, including atrial fibrillation, HF [28,29], and various aortopathies [30,31]. Moreover, it suggests that patients with mild-to-moderate (vs up-to-mild) MR or TR may, under certain circumstances, benefit from interventional treatment of these valvulopathies at the time of the AV surgery. While this last notion is inherently hypothetical at present and not supported by current guidelines [32–35], it should be noted that the latter are based on studies that have stemmed from different populations than ours, namely patients undergoing SAVR for AS (in case of MR correction) or mitral valve surgery altogether (in case of TR intervention). Additional, prospective trials could attest or dispute the above-mentioned impressions and help identify and validate criteria for addressing non-significant MR and TR during SAVR that is performed for AR.

Limitations

First, the study's single-center, retrospective design and small sample size, as well as the lack of a central and blinded data adjudication body, may all hamper the generalizability of the results. However, our cohort was one of the largest thus far in relative terms and resembled previously reported registries, therefore enhancing validity. Second, and again owing to the low number of cases and events, our predictive model should be regarded as exploratory, necessitating larger-scale confirmatory studies. Third, baseline structural characteristics of the mitral and tricuspid valves (e.g., annular dimensions) were not uniformly recorded, which prevented their consideration in the analyses. Fourth, imaging parameters were all determined by TTE studies only. However, this represented a well-accepted, real-world practice at the time of the registry, allowed for comparison of baseline and follow-up examinations in a larger subset of patients, and may facilitate the applicability of our findings. Acknowledging the fluctuating nature of regurgitant lesions, as well as the possible under-estimation of AR severity by TTE, we analyzed patients with both moderate-to-severe and severe AR at baseline.

5. Conclusions

In our single-center experience, significant MR or TR developed in one fifth of patients undergoing SAVR for AR by six years after the intervention, was associated with reduced functional capacity and survival, and correlated with baseline clinical and echocardiographic variables, including atrial fibrillation/flutter, symptomatic HF, mild-to-moderate MR or TR, and composite graft use. Further research is needed to validate our findings and assess their implication on the assessment and management of AR patients referred to SAVR both prior to and at the time of operation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm12196280/s1, Supplemental Table S1: Morality Along the Entire Follow-Up Period; Supplemental Table S2: Rates of Moderate or Severe Mitral and/or Tricuspid Regurgitation on the Last Echocardiogram According to Mitral and/or Tricuspid Regurgitation Severity at Baseline; Supplemental Table S3. Univariable Binary Logistic Regression Model for the Primary Outcome.

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Abbreviations

AR	Aortic regurgitation
AV	Aortic valve
HF	Heart failure
MR	Mitral regurgitation
SAVR	Surgical aortic valve replacement
TR	Tricuspid regurgitation

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Effect of Dapagliflozin on Patients with Rheumatic Heart Disease Mitral Stenosis

An Aldia Asrial [1,2,*], Reviono Reviono [1,3], Soetrisno Soetrisno [1,4], Budi Yuli Setianto [1,5], Vitri Widyaningsih [1,6], Ida Nurwati [1,7], Brian Wasita [1,8] and Anggit Pudjiastuti [9]

[1] Doctoral Program of Medical Sciences Department, Faculty of Medicine, Universitas Sebelas Maret, Surakarta 57126, Indonesia
[2] Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Sebelas Maret—Universitas Sebelas Maret Hospital, Surakarta 57126, Indonesia
[3] Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Sebelas Maret—Universitas Sebelas Maret Hospital, Surakarta 57126, Indonesia
[4] Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sebelas Maret—Universitas Sebelas Maret Hospital, Surakarta 57126, Indonesia
[5] Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Gadjah Mada—Dr. Sardjito General Hospital, Yogyakarta 55281, Indonesia
[6] Department of Public Health, Faculty of Medicine, Universitas Sebelas Maret, Surakarta 57126, Indonesia
[7] Department of Biomedical Sciences, Faculty of Medicine, Universitas Sebelas Maret, Surakarta 57126, Indonesia
[8] Department of Pathology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta 57126, Indonesia
[9] Department of Cardiology and Vascular Medicine, Permata Bunda Hospital, Purwodadi 58114, Indonesia
* Correspondence: aaldiaa8@student.uns.ac.id

Abstract: (1) Background: Mitral stenosis is the most common rheumatic heart disease (RHD). Inflammation and fibrosis are the primary pathophysiology, resulting in left atrial stress and dysfunction. Dapagliflozin is a new heart failure treatment with anti-inflammation and anti-fibrosis effects from previous studies. However, the specific role of dapagliflozin in RHD mitral stenosis is unknown. This study aims to investigate (i) the effect of dapagliflozin on biomarkers of fibrosis, NT-pro BNP levels and left atrial function; (ii) the relationship between the changes in fibrosis biomarkers with left atrial function and NT-pro BNP levels. (2) Methods: An open-label randomized study was conducted on 33 RHD mitral stenosis patients divided into a dapagliflozin group which received 10 mg dapagliflozin and standard therapy, and a control group which only received standard therapy. All patients were examined for levels of PICP, MMP-1/TIMP-1 ratio, TGF-β1, NT-proBNP, mitral valve mean pressure gradient (MPG), and net atrioventricular compliance (Cn) pre- and post-intervention. (3) Results: This study found a significant increase in PICP and TGF-β1 and a reduction in the MMP-1/TIMP-1 ratio in the dapagliflozin group and the control group ($p < 0.05$). In the dapagliflozin group, the levels of NT-pro BNP decreased significantly ($p = 0.000$), with a delta of decreased NT-pro BNP levels also significantly greater in the dapagliflozin group compared to the control ($p = 0.034$). There was a significant increase in Cn values in the dapagliflozin group ($p = 0.017$), whereas there was a decrease in the control group ($p = 0.379$). Delta of changes in Cn values between the dapagliflozin and control groups also showed a significant value ($p = 0.049$). The decreased MPG values of the mitral valve were found in both the dapagliflozin and control groups, with the decrease in MPG significantly greater in the dapagliflozin group ($p = 0.031$). There was no significant correlation between changes in the value of fibrosis biomarkers with Cn and NT-pro BNP ($p > 0.05$). (4) Conclusions: This study implies that the addition of dapagliflozin to standard therapy for RHD mitral stenosis patients provides benefits, as evidenced by an increase in net atrioventricular compliance and decreases in the MPG value of the mitral valve and NT-pro BNP levels ($p < 0.05$). This improvement was not directly related to changes in fibrosis biomarkers, as these biomarkers showed ongoing fibrosis even with dapagliflozin administration.

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1. Introduction

Rheumatic heart disease (RHD) remains a significant health problem that causes mortality and morbidity, mainly in developing countries [1]. Cardiac inflammation and fibrosis of valves and myocardium are the primary manifestations. The disease begins with acute rheumatic fever and molecular mimicry between streptococcal group-A antigen and host tissue, causing activation of immune cells and leading to fibrosis and dysfunction of the valves [2,3]. Several serum biomarkers have been studied and shown to be associated with cardiac fibrosis in RHD. These fibrosis biomarkers include transforming growth factor-β1 (TGF-β1)—a marker of collagen synthesis and extracellular matrix remodeling [4,5]; circulating carboxy-terminal propeptide of type I procollagen (PICP)—a marker of type I collagen synthesis; and the ratio between matrix metalloproteinase I (MMP-1) and tissue matrix metalloproteinase inhibitors 1 (TIMP-1), which describes the balance of collagen degradation processes and inhibition [6,7]. The shift from degradation to synthesis of the extracellular matrix will determine the increase or decrease of collagen and the degree of fibrosis that occurs [8].

Fibrosis of the valve will cause valve dysfunction; one of the most common abnormalities is mitral stenosis [9,10]. In mitral stenosis, there is a disturbance in the opening of the mitral valve, which increases left atrial (LA) and pulmonary pressure and causes complaints of heart failure. In the long term, this pressure will also cause fibrosis in the left atrium. In addition to pressure factors, chronic inflammation of RHD is a stress responsible for left atrial fibrosis [11,12]. This fibrosis then causes interference in the left atrium, which can be measured using the parameter of net atrioventricular compliance (Cn). This parameter has been associated with prognosis after intervention, given its relation to pulmonary hypertension, activity intolerance and progression of mitral stenosis in medical treatments [13]. Stress on the myocardium, especially in the left atrial, also increases the level of NT-pro BNP. This biomarker has been extensively studied in heart failure patients and associated with parameters of left atrial dimension and pressure, the mitral valve area, and patient functional class in RHD mitral stenosis [14,15].

Dapagliflozin is an SGLT2 inhibitor-class drug currently used across a broad spectrum of heart failure cases [16]. Various mechanisms of these drug benefits continue to be studied, including in inflammation and fibrosis pathways [17]. The role of the SGLT2 pathway has been proven in cardiac fibrosis mainly through the collagen type I and III expression pathways found in both in vivo and in vitro studies [18–20]. Research on animal models of mitral regurgitation found that dapagliflozin improves left ventricular cardiac fibrosis [21]. In left atrial clinical studies, dapagliflozin was found to improve left atrial function and maximal volume and reduce the risk of atrial fibrillation/atrial flutter, which is known to be associated with atrial fibrosis [22–25].

Currently, no treatment for rheumatic heart disease mitral stenosis targets the primary pathogenesis—fibrosis. Previous studies have tried several drugs to inhibit fibrosis. However, the results are inconsistent, and these drugs have not yet become standard therapy [26–28]. Therefore, new approaches and treatments are needed to prevent RHD progression, and perhaps to improve LA function. The role and benefits of dapagliflozin in RHD mitral stenosis patients are not known, specifically in fibrosis pathways and left atrial function. This study aims to investigate (i) the effect of dapagliflozin on biomarkers of fibrosis, left atrial function and NT-pro BNP levels and (ii) the relationship between the changes in fibrosis biomarkers with left atrial function and NT-pro BNP levels.

2. Materials and Methods

2.1. Study Design

This study is a clinical experimental study with an open-label design, randomized, controlled trial, pre-test, and post-test design. The protocol was approved by the Faculty of Medicine Universitas Sebelas Maret Research Ethics Committee (No.128/UN27.06.11/KEP/EC.2022). The study was registered in ClinicalTrials.gov (NCT05618223). The sample was randomly divided into two groups (random assignment), namely, the dapagliflozin group (dapagliflozin and standard treatment) and the control group (standard treatment only). Subjects in the dapagliflozin group received standard medical treatment plus dapagliflozin, 10 mg/day for 4 weeks, while subjects in the control group received standard medical treatment only.

2.2. Subject

The study was conducted at Panti Rahayu Hospital and Permata Bunda Hospital in Purwodadi, Indonesia. The subjects included were outpatients at the Cardiology polyclinic for a primary diagnosis of mitral stenosis RHD. This diagnosis was screened with the following inclusion criteria: planimetry mitral valve area ≤ 1.5 cm^2 in echocardiography with morphology supporting RHD (calcification and fusion of leaflets and commissures and with restrictive valve mobility) [29–31]; and New York Heart Association functional class 2–3.

Exclusion criteria included significant (moderate to severe) mitral and aortic valve disease besides mitral stenosis; patients who were pregnant or breastfeeding; patients who were hemodynamically unstable or experiencing severe acute decompensation characterized by signs of congestion in the form of crackles of more than one-third of the lung fields, ascites, and/or signs and symptoms of cardiogenic/hypovolemic shock; patients after mitral valve replacement surgery or after percutaneous balloon mitral valvuloplasty; patients known to be allergic to SGLT2 inhibitors; type 1 diabetes mellitus; patients currently undergoing treatment with SGLT2 inhibitors or having received SGLT2 inhibitor therapy in the last 4 weeks; patients with a history of more than one episode of severe hypoglycemia (GDS < 60 mg/dl) on insulin or sulfonylurea treatment; patients with chronic kidney disease stage IV (estimated glomerular filtration rate (eGFR) = 15–29 mL/min/1.73 m^2) and/or stage V (eGFR < 15 mL/min/1.73 m^2) and/or who are undergoing dialysis (hemodialysis); and patients with severe lung disease.

2.3. Measurement of Biomarker and LA Function

The venous blood sample of each subject was collected into a separate serum tube pre- and post-intervention. The research protocol of TGF-β1, PICP, MMP-1, TIMP-1 and NT-pro BNP levels used the ELISA method and was conducted per the manufacturer's instructions. The ELISA kits used were: Elikine^{TM} Human TGF-β1 ELISA Kit (KET6030) (Abbkine, Atlanta, GA, USA), ABclonal Human PICP chain ELISA Kit (RK09063) (Abclonal, Woburn, MA, USA), ABclonal Human Total MMP-1 ELISA Kit (RK00340), Elikine^{TM} Human TIMP ELISA Kit (KET6031), ABclonal Human NT-pro BNP ELISA Kit (RK09256). The fibrosis biomarkers and NT-pro BNP examination were carried out in the biomedical laboratory of Sebelas Maret University.

Each well contained 100 μL of standard and human serum incubated for 2 h at 37 °C. After washing three times, 100 μL working biotin conjugate antibody was added to the well and set for 1 h at 37 °C. Then, each well received 100 μL working streptavidin-HRP, 90 μL substrate solution and 50 μL stop solution. The final step was to detect optical density within 5–30 min at a wavelength under 450 nm. All standard equipment, including well, microplate reader, multi-channel pipette, incubator, precision pipettes and water, was provided by the biomedical laboratory of Sebelas Maret University.

Standard echocardiography examination to evaluate RHD mitral stenosis was performed pre- and post-intervention.

Left atrial function assessment was performed using an echocardiography General Electric Echocardiography Vivid T8 machine. Each patient was examined by standard

echocardiography to evaluate mitral stenosis, and left atrial function was evaluated by measuring the net atrioventricular compliance value and mitral-valve mean pressure gradient. Net atrioventricular compliance was calculated using the following formula:

$$\text{Cn (mL/mmHg)} = -1270 \times \text{mitral valve area (MVA) (cm}^2\text{)/E-wave downslope (cm/s}^2\text{)}.$$

The value of the mitral valve area was obtained from an echocardiographic examination, using the planimetry method on a parasternal short-axis view at the level of the mitral valve [32,33]. The E-wave downslope value was obtained from an echocardiographic examination using a pulsed wave Doppler at the apical 4-chamber view. In patients with atrial fibrillation, a pulsed-wave Doppler examination was performed 5 times (5 cardiac cycles), and the E-wave downslope value was the average value of the 5 cardiac cycles [34]. After obtaining the mitral valve area and e-wave downslope values, Cn was calculated manually. Mitral valve MPG examination was performed using echocardiography by placing a marker on the tip of the mitral valve in 4-chamber view. Volume sampling was carried out using a continuous wave Doppler to obtain the MPG value of the mitral valve [31].

2.4. Statistical Analysis

The normality test was conducted on each data element to see the distribution. The normality test used the Shapiro–Wilk test, with $p > 0.05$ indicating normal data distribution. For normally distributed data, the test for different means of pre- and post-intervention values in one group was carried out by a paired *t*-test. An unpaired *t*-test was carried out to test differences in means between groups. For data that were not normally distributed, a different test of the means of pre- and post-intervention values in one group was performed by the Wilcoxon signed rank test, while the Mann–Whitney test was carried out to test different means between groups. The correlation between two continuous variables was measured by Pearson's correlation test. The *p*-value is considered significant if it is less than 0.05. Data analysis was performed using IBM® SPSS® statistics version 25.

3. Results

Thirty-three patients were enrolled (17 patients in the dapagliflozin group and 16 in the control group), with similar baseline characteristics (Table 1).

Table 1. Group characteristics and baseline data.

Patients	Dapagliflozin Group (*n* = 17)	Control Group (*n* = 16)	*p*-Value
Demography and comorbidities			
Age, years	51.35 ± 9.88	55.94 ± 6.65	0.13
Sex			
Female, *n* (%)	14 (82.53%)	15 (93.75%)	0.601
Male, *n* (%)	3 (17.64%)	1 (6.25%)	
Body mass index (BMI), kg/m^2	22.87 ± 3.14	21.08 ± 3.10	0.11
Atrial fibrillation (%)	17 (100%)	16 (100%)	-
Hypertension, *n* (%)	1 (5.88%)	3 (1.87%)	0.335
Type 2 diabetes, *n* (%)	2 (11.76%)	0 (0%)	0.485
Coronary artery disease, *n* (%)	0 (0%)	0 (0%)	-
Smoker, *n* (%)	1 (5.88%)	1 (6.25%)	0.965
Examination			
Systolic blood pressure, mmHg	118.53 ± 13.25	116.63 ± 17.25	0.417
Diastolic blood pressure, mmHg	76.71 ± 10.83	78.00 ± 13.79	0.766
Heart rate, bpm	72.11 ± 14.13	78.93 ± 15.50	0.196
Creatinine, mg/dL	1.03 ± 0.48	0.93 ± 0.34	0.773
eGFR, mL/ mL/min/1.73 m^2	67.94 ± 26.37	65.37 ± 22.22	0.765
Blood glucose, mg/dL	128.94 ± 43.78	116.75 ± 22.22	0.787

Table 1. *Cont.*

Patients	Dapagliflozin Group (*n* = 17)	Control Group (*n* = 16)	*p*-Value
Echocardiography parameters			
MVA planimetry, cm^2	0.75 ± 0.13	0.77 ± 0.13	0.616
LA diameter, mm	56.14 ± 9.26	53.52 ± 8.36	0.402
RV diameter, mm	36.29 ± 4.51	33.46 ± 7.09	0.177
LVIDd, mm	47.34 ± 7.21	45.99 ± 5.81	0.665
LAVI, mL/m^2	145.89 ± 75.29	139.26 ± 69.27	0.707
Cn, mLmmHg	4.82 ± 1.71	5.21 ± 1.99	0.546
Mean pressure gradient mitral, mmHg	13.23 ± 4.50	12.35 ± 4.48	0.579
Systolic pulmonary artery pressure, mmHg	64.83 ± 14.74	65.65 ± 22.79	0.902
LVEF, %	53.73 ± 10.36	57.20 ± 9.02	0.314
TAPSE, mm	18.30 ± 4.12	18.42 ± 7.53	0.954
Pulmonary hypertension probability (intermediate to high), %	15 (88.23%)	12 (75%)	0.398
Pharmacological treatment			
ACE-I/ ARB	0 (0%)	0 (0%)	-
Beta blockers	12 (70.58%)	8 (50%)	0.394
Furosemide	13 (76.47%)	12 (75%)	1
Spironolactone	17 (100%)	16 (100%)	-
Antiplatelet	0 (0%)	0 (0%)	-
Warfarin	17 (100%)	16 (100%)	-
Digoxin	5 (29.41%)	8 (50%)	0.394
Complication			
Hypoglycemia	1 (5.88%)	0 (0%)	0.303
Diabetic ketoacidosis	0 (0%)	0 (0%)	-
Hypotension	1 (5.88%)	0 (0%)	0.303
Amputation	0 (0%)	0 (0%)	-
Genital infection	0 (0%)	0 (0%)	-

3.1. Effect of Dapagliflozin on Biomarker Fibrosis Levels in RHD Mitral Stenosis

This study found a significant increase in PICP and TGF-β1 values post-intervention in the dapagliflozin and control groups ($p = 0.000$). Meanwhile, in the MMP-1/TIMP-1 ratio, there was a significant decrease in the dapagliflozin group ($p = 0.005$) and the control group ($p = 0.002$). The delta changes of PICP, TGF-β1, and the MMP-1/TIMP-1 ratio were not significantly different between the dapagliflozin and control groups. These results confirmed that the fibrosis process was still ongoing, and that administration of dapagliflozin had not been shown to inhibit the increase in PICP and TGF-β1 and decrease the MMP-1/TIMP-1 ratio (Table 2).

Table 2. Effect of dapagliflozin on fibrosis biomarkers PICP, MMP-1/TIMP-1 ratio and TGF-β1.

	PICP (ng/mL)			MMP-1/TIMP-1 Ratio			TGF-β1 (pg/mL)		
	Dapagliflozin Group	Control Group	*p*-Value	Dapagliflozin Group	Control Group	*p*-Value	Dapagliflozin Group	Control Group	*p*-Value
Pre-intervention	67.23 ± 44.61	56.04 ± 22.66	0.614	0.63 ± 0.31	0.73 ± 0.75	0.540	1.66 ± 0.64	1.39 ± 0.49	0.195
Post-intervention	158.54 ± 71.18	161.45 ± 107.63	0.719	0.32 ± 0.23	0.23 ± 0.16	0.171	3.38 ± 2.26	2.25 ± 1.77	0.058
p-value (post-pre)	0.000	0.000		0.005	0.002		0.005	0.044	
Delta (Δ)	91.30 ± 59.83	105.41 ± 94.88	0.943	0.31 ± 0.35	0.50 ± 0.68	0.885	1.73 ± 2.34	0.86 ± 1.77	0.207

3.2. Effect of Dapagliflozin on NT-pro BNP Levels in RHD Mitral Stenosis

Results indicating positive effects of dapagliflozin were obtained as to NT-pro BNP levels. There was a significant reduction in NT-pro BNP levels in both the dapagliflozin and

control groups. In the dapagliflozin group, the levels of NT-pro BNP decreased significantly, from 7045.29 ± 3182.26 pg/mL to 3210.88 ± 1019.46 pg/mL (p = 0.000). In the control group, the level of NT-pro BNP also decreased significantly, from 6928.12 ± 3690.44 pg/mL to 4971.87 ± 3634.65 mg/dL (p = 0.002). A significant difference in NT-pro BNP levels was also found in the delta of decreased NT-pro BNP levels in the treatment group compared to the control (3832.42 ± 2857.52 vs 1956.25 ± 1755.42; p = 0.034). From the results of this analysis, it was found that dapagliflozin and standard medication significantly reduced levels of NT-pro BNP. Even so, dapagliflozin administration reduced NT-pro BNP levels more than the reduction in the control group (Table 3 and Figure 1).

Table 3. Effect of dapagliflozin on NT-pro BNP levels.

	Mitral-Valve Mean Pressure Gradient (pg/mL)		*p*-Value
	Dapagliflozin Group	**Control Group**	
Pre-intervention	7045.29 ± 3182.26	6928.12 ± 3690.44	0.857
Post-intervention	3210.88 ± 1019.46	4971.87 ± 3634.65	0.449
p-value (post-pre)	0.000	0.002	
Delta (Δ)	3832.42 ± 2857.52	1956.25 ± 1755.42	0.034

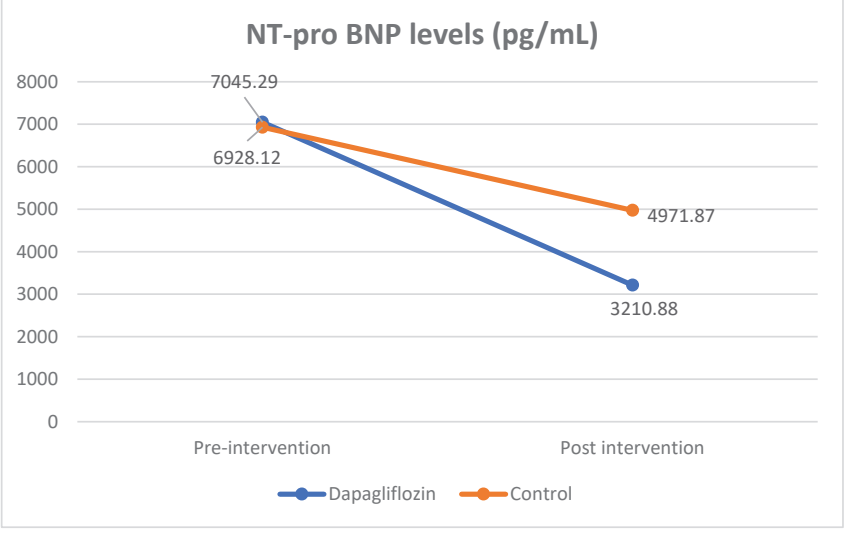

Figure 1. Comparison of NT-pro BNP levels pre- and post-intervention.

3.3. Effect of Dapagliflozin on Cn and Mitral Valve Mean Pressure Gradient in RHD Mitral Stenosis

We found no significant differences in echocardiographic parameters in the dapagliflozin and control groups pre- and post-intervention. When analyzing the differences in parameters within each group, we found no significant differences except for the net atrioventricular compliance and mitral valve mean pressure gradient parameters. There was a reduction in RV diameter and LAVI, but it was not statistically significant (Table 4).

Although the fibrosis biomarkers did not show inhibition of the fibrotic process, we found an increase in left atrial function, as measured by the Cn value. There was a significant increase in Cn values in the post-intervention dapagliflozin group ((4.82 ± 1.71 to 5.73 ± 2.19 mL/mmHg; p = 0.017). In the control group, there was a decrease in post-intervention Cn values (5.21 ± 1.99 to 4.68 ± 1.73 mL/mmHg; p = 0.379). There was a

significant difference in the delta changes in Cn values between the dapagliflozin and control groups ($p = 0.049$) (Table 5 and Figure 2).

Table 4. Echocardiography parameters pre- and post-intervention.

Echocardiography Parameters	Dapagliflozin Group ($n = 17$)			Control Group ($n = 16$)			*p*-Value (between Pre-Intervention Group)	*p*-Value (between Post-Intervention Group)
	Pre-Intervention	Post-Intervention	*p*	Pre-Intervention	Post-Intervention	*p*		
MVA planimetry, cm^2	0.75 ± 0.13	0.73 ± 0.03	0.403	0.77 ± 0.13	0.72 ± 0.03	0.099	0.616	0.905
LA diameter, mm	56.14 ± 9.26	56.06 ± 1.84	0.529	53.52 ± 8.36	53.72 ± 2.11	0.702	0.402	0.952
RV diameter, mm	36.29 ± 4.51	34.58 ± 4.95	0.057	33.46 ± 7.09	34.09 ± 1.14	0.679	0.177	0.822
LVIDd, mm	47.34 ± 7.21	49.34 ± 6.21	0.091	45.99 ± 5.81	45.14 ± 5.91	0.275	0.665	0.113
LAVI, mL/m^2	145.89 ± 75.29	139.26 ± 69.27	0.055	139.26 ± 69.27	149.63 ± 105.89	0.326	0.707	0.744
Cn, mL/mmHg	4.82 ± 1.71	5.73 ± 2.19	0.017	5.21 ± 1.99	4.68 ± 1.73	0.379	0.546	0.121
Mean pressure gradient mitral, mmHg	13.23 ± 4.50	9.88 ± 3.87	0.001	12.35 ± 4.48	11.88 ± 4.01	0.756	0.579	0.155
Systolic pulmonary artery pressure, mmHg	64.83 ± 14.74	67.89 ± 9.78	0.446	65.65 ± 22.79	73.49 ± 22.32	0.335	0.902	0.353
LVEF, %	53.73 ± 10.36	50.31 ± 8.75	0.215	57.20 ± 9.02	56.02 ± 6.96	0.682	0.314	0.046
TAPSE, mm	18.30 ± 4.12	17.51 ± 3.76	0.568	18.42 ± 7.53	18.00 ± 4.22	0.480	0.954	0.423
Pulmonary hypertension probability (intermediate to high), %	15 (88.23%)	14 (82.35%)	1.00	12 (75.00%)	11 (64.71%)	1.00	0.398	0.362

Table 5. Effect of dapagliflozin on Cn value.

	Cn (mL/mmHg)		*p*-Value
	Dapagliflozin Group	Control Group	
Pre-intervention	4.82 ± 1.71	5.21 ± 1.99	0.546
Post-intervention	5.73 ± 2.19	4.68 ± 1.73	0.121
p-value (post-pre)	0.017	0.379	
Delta (Δ)	0.90 ± 1.29	−0.53 ± 2.57	0.049

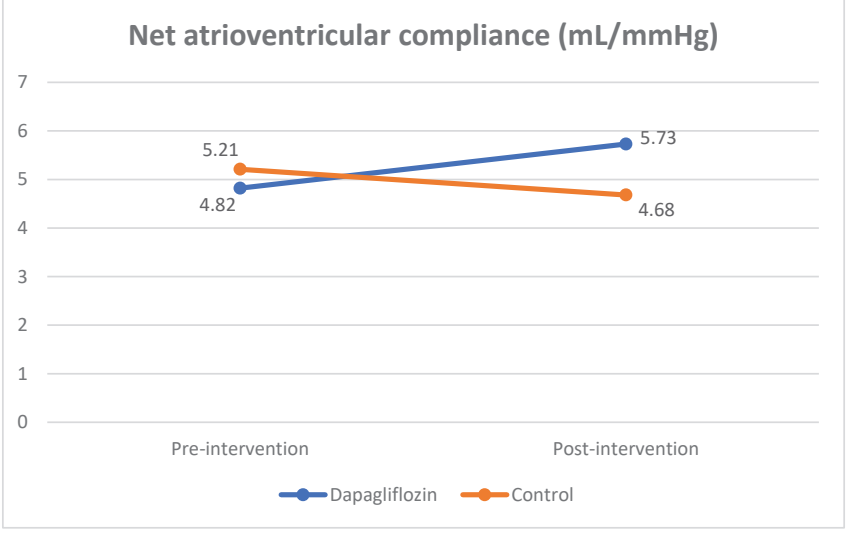

Figure 2. Comparison of net atrioventricular compliance (Cn) value pre- and post-intervention.

The mitral valve mean pressure gradient parameter also showed a significant improvement in the dapagliflozin group, as compared to the control group. A decrease in the MPG

value of the mitral valve was found in both the dapagliflozin group and the control group (3.34 ± 3.11 and 0.46 ± 4.15 mmHg), but the decrease in MPG was significantly greater in the dapagliflozin group ($p = 0.031$ (Table 6).

Table 6. Effect of dapagliflozin on MPG mitral valve.

	Mitral Valve Mean Pressure Gradient (mmHg)		*p*-Value
	Dapagliflozin Group	Control Group	
Pre-intervention	13.23 ± 4.50	12.35 ± 4.48	0.579
Post-intervention	9.88 ± 3.87	11.88 ± 4.01	0.155
p-value (post-pre)	0.001	0.756	
Delta (Δ)	3.34 ± 3.11	0.46 ± 4.15	0.031

In subsequent analyses, we found a significant association between change in Cn values and mitral valve MPG (r = −0.463; $p = 0.007$). Better Cn is associated with lower mitral-valve MPG values (Figure 3).

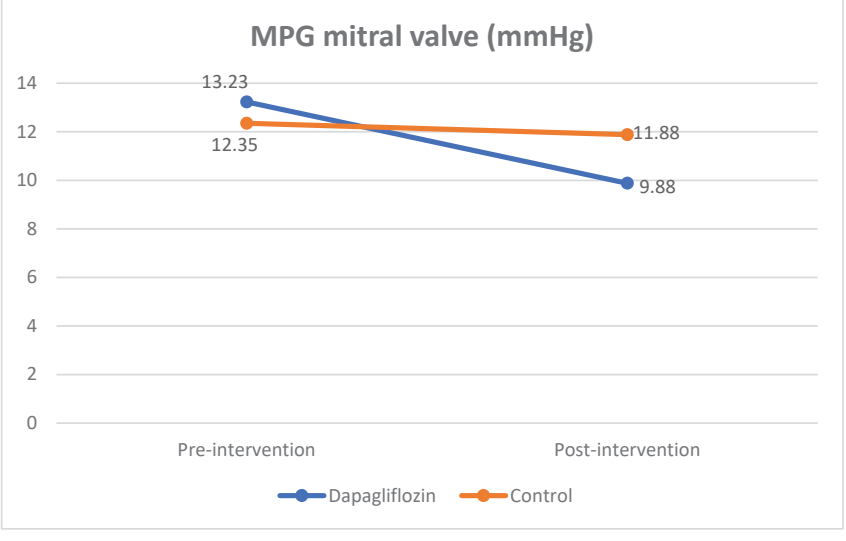

Figure 3. Comparison of MPG mitral-valve value pre- and post-intervention.

3.4. Relationship of Changes in Fibrosis Biomarkers with Cn and NT-pro BNP Levels

In this study, we did not find a significant correlation between changes in the values of the biomarkers PICP, MMP-1/TIMP-1 ratio, or TGF-β1 with Cn (PICP with Cn (r = −0.297; $p = 0.093$); MMP-1/TIMP-1 with Cn (r = −0.056; $p = 0.756$); TGF-β1 with Cn (r = 0.057; $p = 0.751$)).

There was also no correlation between changes in fibrosis biomarker values and NT-pro BNP levels (PICP with NT-pro BNP (r = −0.240; $p = 0.354$), MMP-1/TIMP-1 ratio with NT-pro BNP (r = 0.330; $p = 0.196$); TGF-β1 with NT-pro BNP (r = −0.302; $p = 0.238$)).

4. Discussion

The anti-fibrosis effects of dapagliflozin have been demonstrated in several studies. Ye et al. (2017) found that dapagliflozin attenuated the activation of the inflammasome, fibrosis, and deterioration of LVEF in BTBR mice model cardiomyopathy. Dapagliflozin significantly attenuated the elevated mRNA levels of NALP3, ASC, IL-1β, IL-6, caspase-1, and TNFα in the BTBR mice model. Then, dapagliflozin also significantly attenuated the

increase in type I and type III collagen mRNA levels and reduced the percentage of fibrosis on Masson's trichrome staining [35].

Activation of the TGFβ1/Smad signaling pathway is one of the main pathways of cardiac fibrosis. Research by Zhang et al. (2021) found that administration of dapagliflozin inhibited cardiac fibroblast (CF) collagen production induced by angiotensin II in vitro by regulating TGF-β1/Smads signaling. Dapagliflozin pretreatment inhibited left ventricular dysfunction, left ventricular hypertrophy, fibrosis, and collagen synthesis induced by angiotensin II [20]. Meanwhile, in a study by Chen et al. (2022), inhibition of this pathway by dapagliflozin reduced the expression of MMP-2, MMP-9 and TIMP-1 ($p < 0.05$), thereby improving fibrosis in normoglycemic heart failure rabbit models [36].

However, the anti-fibrotic effects of dapagliflozin were not proved in this study. The treatment group with dapagliflozin did not show significant inhibition in increasing PICP and TGF-β1 levels and decreasing the MMP-1/TIMP-1 ratio. There are several possibilities to explain why this inhibitory effect was not proved in this study. Dapagliflozin has improved fibrosis through the TGFβ1/Smad signaling pathway, the NLRP3/ASC inflammasome, or the mitogen-activated protein kinase (MAPK) signaling pathway [18–20,35,36]. Nevertheless, the fibrosis signaling pathway for rheumatic heart disease is still being studied, and other pathways may play a role beyond those already known. A previous review by Xian and Zheng (2021) has identified three intervention targets that can be used for the treatment of RHD: interventions in IFN-γ and TNF-α--mediated ECM remodeling, suppression of α-SMA expression in TGF-β1-induced fibroblasts via the AKT/S6K pathway and disruption of STAT3 phosphorylation to prevent cytokine release from Th17 cells and reduce induction of valve damage by RHD. However, it is not yet known how much impact intervention in that specific pathway will have on fibrosis in RHD mitral stenosis, and further study is needed [37].

This study is also based on several previous studies related to the use of dapagliflozin in human heart failure patients, in which significant clinical benefits have been seen on the 28th day [38]. Even so, specifically regarding the intervention for fibrosis by administering dapagliflozin, several animal studies were carried out over a more extended period [19,35]. The duration of dapagliflozin use and the emergence of anti-inflammatory and anti-fibrosis effects in humans are unknown. A longer intervention time may be needed to see the impact of dapagliflozin on biochemical marker parameters of fibrosis, although left atrial function has shown significant changes.

The increase in fibrosis biomarkers can be caused by the fibrosis process in the valves and myocardium atrial and ventricles. Valves are structurally distinct from the myocardium, including their response to inflammation and fibrosis in RHD. The fibrotic response in the valves is more severe and causes permanent damage. One of the hypotheses that explain this valve damage refers to the lower level of anti-inflammatory cytokine (IL-4) in the valve compared to the myocardium [39,40]. Thus far, research on dapagliflozin referencing heart disease has mainly been carried out to target the myocardium. The biochemical markers of fibrosis used in this study are circulating markers whose increase can occur by the increasing fibrosis of either the valves or the myocardium.

Sodium–glucose transporters mediate apical sodium and glucose transport across cell membranes and are also known as Na^+/glucose co-transporters or symporters (SGLTs). SGLT2 is a member of the SLC5 gene family, a subdivision of an ancient superfamily of sodium co-transporters [41]. Until now, the expression of SGLT2 receptors in valvular areas has been unknown. SGLT2 is mainly expressed in the kidney, and is located in the first part of the proximal tubule, which allows ∼90% of glucose reabsorption from the urine. The SGLT2 receptors have not been detected in cardiomyocytes but are known to directly affect the heart [42].

The mechanism of action of dapagliflozin in heart failure is still a question, and the research is continuing. In addition to the inflammatory and fibrotic pathways, there are several other hypotheses, such as their effect on cardiac metabolism and myocardial bioenergetics, changes in adipokines and epicardial adipose tissue mass, as well as their

impact on loading conditions mainly through the natriuresis–diuresis pathway [43]. A comparative study by Wilcox et al. (2018) [44] found that dapagliflozin has the same sodium-reducing effect and interstitial fluid volume as the loop diuretic bumetanide but without a significant change in intravascular volume. Another study by Heerspink et al. (2013) [45] found that giving dapagliflozin for 12 weeks compared to hydrochlorothiazide reduced plasma volume and increased erythrocyte mass. Interstitial volume regulation is important for patients with heart failure, including patients with RHD mitral stenosis. Compared to conventional diuretic drugs, which cause a decrease in interstitial and intravascular volume, the selective effect of dapagliflozin on interstitial volume without interference with intravascular volume will be beneficial. This selective effect does not cause reflex neurohumoral stimulation, which can exacerbate heart failure [43–45]. Fluid volume is an essential component of left atrial function. At the same MVA value, MPG will be directly proportional to the fluid volume and inversely proportional to the filling time of the ventricles in the diastole phase [46]. Fluid volume, left atrial volume, trans-mitral blood flow (mean pressure gradient mitral valve) and Cn are interrelated factors associated with left atrial mechanical dysfunction [47,48].

Regarding the role of dapagliflozin in improving cardiac chamber pressure, especially the left atrium, similar results were obtained in the phase II randomized clinical trial "Evaluation of the Cardiac and Metabolic Effects of Dapagliflozin in Heart Failure with Preserved Ejection Fraction" (CAMEO-DAPA). In this study, dapagliflozin was administered to heart failure patients with preserved ejection fraction. During the 24-week observation period, a significant decrease in pulmonary capillary wedge pressure (PCWP) was found, reflecting left atrial pressure in the dapagliflozin treatment group either at rest (Δ absolute difference: –3.5 mmHg; 95% CI: –6.7 to –0.4; $p = 0.029$) or during activity (Δ absolute difference: –6.1 mmHg; 95% CI: –11.2 to –1.0; $p = 0.019$). Dapagliflozin also significantly reduced right atrial and pulmonary artery pressure during activity, plasma volume and body weight [49]. Another research trial on the "Impact of Dapagliflozin on Left Ventricular Diastolic Dysfunction in Patients with Type 2 Diabetes Mellitus" (IDDIA) also showed the dapagliflozin effect on left ventricular pressure. Dapagliflozin administration in type 2 diabetes patients with standard therapy was associated with significantly improved left ventricular diastolic function and decreased estimated LV filling pressure on exercise [50]. Another mechanism that can explain the improvement of myocardial function is the ion pathway and endothelium function. Research by Cappetta et al. [51] in Dahl rats showed that dapagliflozin reduced Ca^{2+} and Na^{+} overload and prevented decreased Ca^{2+} transient amplitude. Dapagliflozin was also found to improve endothelial function, as evidenced by a decrease in markers of endothelial activation. Dapagliflozin was further shown to partially restore endothelial nitric oxide synthase, which was downregulated in diastolic dysfunction ($p < 0.05$) [51].

We also found a significant decrease in NT-pro BNP levels in the dapagliflozin group. The NT-pro BNP value describes the level of myocardial stress; in previous studies, the NT-pro BNP value has been correlated with echocardiography parameters and the patient's functional class [15]. The study by Iltumur et al. (2005) also found that NT-pro BNP levels correlated positively with the severity of mitral stenosis and pulmonary artery pressure and negatively correlated with the mitral valve area (MVA) ($p < 0.001$) [14]. The NT-pro BNP value can also explain the relationship between hemodynamic status and patient symptoms, so this parameter can be used to monitor the progression and clinical severity of RHD mitral stenosis [52]. The role of NT-pro BNP is also influenced by the management carried out; in the study of Safi et al. (2017), a significant decrease in NT-pro BNP was found after the percutaneous mitral commissurotomy intervention in RHD mitral stenosis patients, and the decrease in value correlated with a decrease in the mean pressure gradient (MPG) [53].

Based on several previous studies, the ventricular myocardium is known to be the main source of BNP. However, other studies have uncovered the possibility of different sites synthesizing or producing BNP. Research by Khare and Dwivedi (2016) found a correlation

between left atrial dysfunction examined by tissue-Doppler-derived strain/strain rate (S/Sr) and NT-pro BNP levels [54]. This hormone level can also be used to predict improvement in left atrial function after percutaneous mitral balloon valvuloplasty. In another study with lone atrial fibrillation patients, blood samples taken from the coronary sinus showed levels of NT-pro BNP higher than those of the aorta and anterior interventricular vein (AIV), where samples from the coronary sinus indicated an NT-pro BNP value in the atrium [55].

This finding was supported by a study by Sharma et al. (2011) [56] in RHD mitral stenosis patients; it was found that both BNP and atrial natriuretic peptide (ANP) were associated with disease severity, but ANP was not shown to be significantly related to exercise capacity or increased blood pressure during exercise. In contrast, increased BNP was associated with a lower left atrial area index, lower exercise capacity, and higher pulmonary artery pressure [54]. A decrease in the value of NT-pro BNP in our study could indicate a decrease in myocardial stress, specifically in the left atrial myocardium [56].

In general, these results open up new potential beneficial effects of dapagliflozin administration in patients with RHD mitral stenosis. It is hoped that improving Cn function, decreasing MPG of the mitral valve, and NT-pro BNP levels will help reduce signs and symptoms of heart failure in patients.

There are several limitations to this research. This research was conducted on a small sample size as a preliminary study. The intervention period was also short, so in the future, it could be carried out on a larger number of subjects and for a longer duration. In addition, the biomarkers of fibrosis that were examined are biomarkers circulating in the circulation, so there is still the possibility of being influenced by other factors; thus, an assessment with other methods is needed to assess fibrosis in the left atrium, for example, with the Cardiac magnetic resonance imaging (CMR). Other mechanisms related to improving left atrial function besides the fibrosis pathway also need to be investigated to find out the mechanism for the improvement of left atrial function in this study, even though no inhibition was found in the fibrosis process. Further in-depth research is needed to understand the mechanism of dapagliflozin's beneficial effect in RHD mitral stenosis patients.

5. Conclusions

Administration of dapagliflozin in RHD mitral stenosis patients has been shown to improve left atrial function, as evidenced by improvements in Cn, the MPG value of the mitral valve, and NT-pro BNP levels. This improvement was not directly related to changes in fibrosis biomarkers, as these biomarkers showed ongoing fibrosis, even with dapagliflozin administration.

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Targeted Radiation Exposure Induces Accelerated Aortic Valve Remodeling in ApoE$^{-/-}$ Mice

Guillaume Rucher [1,†], Kevin Prigent [1,2], Christophe Simard [1], Anne-Marie Frelin [3], Maëlle Coquemont-Guyot [4], Nicolas Elie [4], Nicolas Delcroix [5], Nicolas Perzo [6], Romain Guinamard [1], Ludovic Berger [1,7], Alain Manrique [1,2,*] and on behalf of the STOP-AS Investigators [‡]

1 Normandie Univ, UNICAEN, UR 4650 PSIR, GIP Cyceron, 14000 Caen, France; kevin.prigent.lp@gmail.com (K.P.); christophe.simard@unicaen.fr (C.S.); romain.guinamard@unicaen.fr (R.G.); berger-l@chu-caen.fr (L.B.)
2 Department of Nuclear Medicine, CHU de Caen, 14000 Caen, France
3 Grand Accélérateur National d'Ions Lourds (GANIL), CEA/DRF-CNRS/IN2P3, 14000 Caen, France
4 Normandie Univ, UNICAEN, SF 4207, PLATON Services Unit, Virtual'His, 14000 Caen, France; nicolas.elie@unicaen.fr (N.E.)
5 CNRS, UMS-3048, GIP Cyceron, Campus Jules Horowitz, 14000 Caen, France
6 Normandie Univ, UNIROUEN, INSERM U1096 EnVI, 76000 Rouen, France; nperzo@gmail.com
7 Department of Vascular Surgery, Normandie Univ, UNICAEN, UR 4650 PSIR, CHU de Caen, 14000 Caen, France
* Correspondence: manrique@cyceron.fr; Tel.: +33-231-470-287
† This Manuscript is the Extension of Guillaume Rucher's Ph.D. Thesis.
‡ Collaborators of the STOP-AS Investigators is provided in the Supplementary Materials.

Abstract: Thoracic radiation therapy may result in accelerated atherosclerosis and in late aortic valve stenosis (AS). In this study, we assessed the feasibility of inducing radiation-induced AS using a targeted aortic valve irradiation (10 or 20 Grays) in two groups of C57Bl6/J (WT) and ApoE$^{-/-}$ mice compared to a control (no irradiation). Peak aortic jet velocity was evaluated by echocardiography to characterize AS. T2*-weighted magnetic resonance imaging after injection of MPIO-αVCAM-1 was used to examine aortic inflammation resulting from irradiation. A T2* signal void on valve leaflets and aortic sinus was considered positive. Valve remodeling and mineralization were assessed using von Kossa staining. Finally, the impact of radiation on cell viability and cycle from aortic human valvular interstitial cells (hVICs) was also assessed. The targeted aortic valve irradiation in ApoE$^{-/-}$ mice resulted in an AS characterized by an increase in peak aortic jet velocity associated with valve leaflet and aortic sinus remodeling, including mineralization process, at the 3-month follow-up. There was a linear correlation between histological findings and peak aortic jet velocity ($r = 0.57$, $p < 0.01$). In addition, irradiation was associated with aortic root inflammation, evidenced by molecular MR imaging ($p < 0.01$). No significant effect of radiation exposure was detected on WT animals. Radiation exposure did not affect hVICs viability and cell cycle. We conclude that targeted radiation exposure of the aortic valve in mice results in ApoE$^{-/-}$, but not in WT, mice in an aortic valve remodeling mimicking the human lesions. This preclinical model could be a useful tool for future assessment of therapeutic interventions.

Keywords: aortic stenosis; radiation therapy; mineralization; magnetic resonance imaging

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1. Introduction

Cardiac diseases are a major cause of late mortality and morbidity after mediastinal radiotherapy. The delayed cardiotoxicity may involve all components of the heart, increasing the risk of pericardial disease, cardiomyopathy, arrhythmias, coronary artery disease, and valvular heart disease, including both aortic regurgitation and stenosis [1]. Radiation-induced aortic valve remodeling is characterized by fibrosis and calcification leading to

aortic valve stenosis (AS) and/or regurgitation [1]. Although aortic regurgitation is one of the most common side effects, aortic stenosis is more likely to require an interventional correction [1]. Degenerative AS is a common form of valvular heart disease in developed countries [2], affecting 40% of patients over 80 years old [3]. After mediastinal radiotherapy, the progression of a pre-existing AS is accelerated, further underlining the potential of irradiation to initiate and develop valvular lesions [4]. The same phenomenon is also observed at the vascular level, with an acceleration and/or induction of atherosclerosis in irradiated patients [5].

Previous results have indicated that valve remodeling is mostly due to a direct effect of radiation on valvular cells. The incidence of valvular disease correlates with radiation dose directly delivered to the valve [6]. After an early inflammatory phase occurring within days of irradiation, fibrogenic effector cells can differentiate into myofibroblasts, characterized by collagen and α-smooth muscle actin secretion, which may lead to late fibrosis [7]. We recently observed that transient receptor potential melastatin 4 (TRPM4), a non-selective cation channel involved in the differentiation of human atrial fibroblasts into myofibroblasts [8], is also involved in irradiation-induced aortic valve fibrosis [9]. In addition, recent in vitro experiments demonstrated that irradiation of human aortic valvular interstitial cells (hVICs) induced the expression of osteogenic factors 24 h after irradiation, including bone morphogenetic protein 2 (BMP-2), alkaline phosphatase (ALP), and Runx2 [10]. These phenomena are similar to the early pathophysiology of degenerative AS which involves mechanical lesions, chronic inflammation, and osteogenic phenotypic changes in valve interstitial cells, leading to progressive mineralization [11].

The combination of cardiovascular risk factors and radiation therapy increases radiation-induced peripheral atherosclerosis in humans [12]. The apolipoprotein E-deficient ($ApoE^{-/-}$) mouse is a widely used animal model of atherosclerosis, demonstrating spontaneous atherosclerotic lesions throughout the aortic tree [13] and late aortic valve sclerosis similar to that observed in humans [14]. Based on these findings, we hypothesized that a targeted aortic valve irradiation in $ApoE^{-/-}$ mice may induce an accelerated valve remodeling mimicking human delayed radiation-induced aortic valve stenosis.

2. Materials and Methods

2.1. Animals

The control animals were distributed in 2 groups of C57Bl6/J (group WT RT-, n = 10) and $ApoE^{-/-}$ mice (group $ApoE^{-/-}$ RT-, n = 11). A total of 26 animals were randomly allocated to 10 or 20 Grays (Gy) for both genotypes (WT 10 Gy, n = 6; WT 20 Gy, n = 6; $ApoE^{-/-}$ 10 Gy, n = 4; and $ApoE^{-/-}$ 20 Gy, n = 10). All animals were male and maintained ad libitum with a standard chow diet composed of 8.4% fat, 19.3% protein, 72.4% carbohydrates, 0.55% phosphorus, 0.73% calcium, 0.16% magnesium, and 1000 UI/kg vitamin D3.

2.2. Three-Dimensional Anatomic Atlas of Aortic Valve

To target aortic valve irradiation, we built a murine cardiac atlas using cardiac magnetic resonance (CMR). All CMR experiments were carried out using a 7T magnet (Pharmascan® Bruker, Billerica, MA, USA) under gas anesthesia induced with 5% isoflurane (Forene®, AbbVie, Rungis, France) and maintained with 2% isoflurane in a mix of O_2 and N_2O (1:2). T1 Flash MR images were performed in 13 male C57Bl6/J mice (16 weeks old) using a strict axial multi-slice sequence encompassing the heart: TR/TE 176.6/4.2 ms, 20 slices, 6 repetitions. Automatic alignment and fusion of the 6 repetitions were performed using ImageJ software (version 1.52a, Bethesda, MD, USA) to obtain one single image series for each animal. The aortic valve and the aorta from the insertion to the aortic isthmus were segmented. Then, a threshold set to 75% of co-localization of each aortic valve segmentation was performed to determine the aortic valve segmentation on the atlas.

2.3. In Vivo Irradiation Protocol

All aortic valve irradiations were performed in 16-week-old animals at the EquipHex RecHadron facility (Caen, France), with an X-RAD 225Cx micro-irradiator (Precision X-ray Inc., North Brandord, CT, USA) using fractions of 2.22 Gy/min (225 kV, 13 mA, 0.3 mm copper filter). Anesthesia was induced with 5% isoflurane (Forene®, AbbVie, Rungis, France) and maintained with 2% isoflurane in a mix of O_2 and N_2O (1:2). Animals were placed in prone position on a dedicated bed and computed tomography (CT) encompassing the chest (80 kV, 0.5 mA) was performed. Using 3D Slicer v4.8.1 software (http://www.slicer.org, accessed on 1 June 2018), the segmented aortic valve provided by the cardiac atlas was aligned with CT acquisition using trachea and bronchia bifurcation as anatomical markers. Planning of radiation exposure was performed using SmartPlan® (Precision X-ray Inc., North Brandford, CT, USA). Tissues (air, lung, soft tissue, bone) were segmented using Hounsfield units. Irradiation consisted of 2 beams of 2 mm diameter with an angle of 45° and 315° (Figure 1) in order to avoid both the trachea and the esophagus, after beam spatial resolution and dose distribution were confirmed using polymethyl methacrylate (PMMA) phantoms and gafchromic films.

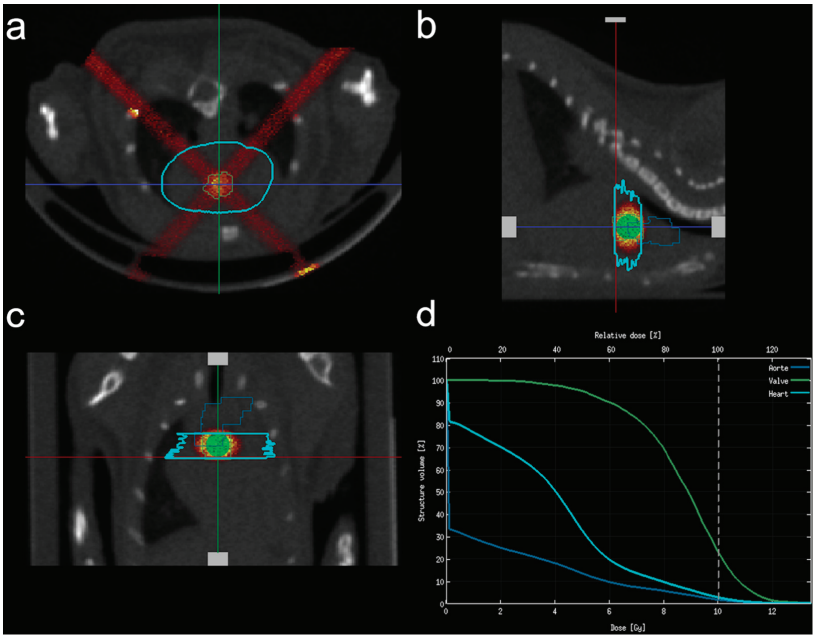

Figure 1. Irradiation plan consisted of 2 X-ray beams of 2 mm diameter with an angle of 45° and 135°. (**a**) Axial view; (**b**) sagittal view; (**c**) frontal view; (**d**) relative dose calculation within the aortic valve (green line), the surrounding heart (light blue) and the aortic arch (dark blue).

2.4. Echocardiography

Echocardiography was performed in isoflurane-anesthetized mice using a iE33 ultrasound system (Philips Healthcare, Best, The Netherlands) and a linear ultrasound probe L15-7io (128 elements, 7–15 MHz). M-mode images of the parasternal long and short axis views were used at baseline and 3-month follow-up to measure left ventricle dimensions. Aortic valve function was assessed using a Doppler measurement of peak aortic jet velocity and mean transvalvular gradient. The mean of 3 consecutive measurements for each parameter was calculated.

2.5. Magnetic Resonance Imaging of Aortic Inflammation

Additional MR experiments were carried out using a 7T magnet (Pharmascan® Bruker, Ettlingen, Germany). End-diastolic MR images were acquired using a multi-slice T2*-weighted sequence encompassing the thoracic aorta in 2 WT RT-, 4 WT 10 Gy, in 3 WT 20 Gy, in 4 ApoE$^{-/-}$ RT-, in 4 ApoE$^{-/-}$ 10 Gy, and in 6 ApoE$^{-/-}$ 20 Gy mice. The acquisition parameters were as follows: field of view: 1809 × 939 mm, slice thickness: 0.15 mm, spatial resolution: 0.1 × 0.1 mm, TR/TE: 100/4.25 ms. Acquisitions were performed before and after intravenous injection of 200 µL of MPIO-αVCAM-1. As previously described [15], microparticles of iron-oxide (DynaBeads MyOnes Tosyl Activated, ThermoFisher Scientific, Waltham, MA, USA) were conjugated with the antibodies anti-αVCAM-1 (clone A(429), BD BioScience, Franklin Lakes, NJ, USA) through incubation at 37 °C for 48 h. MR images were analyzed using Osirix v.6.5.2 software. A T2* signal void on valve leaflets and aortic sinus resulting from MPIO-αVCAM-1 binding was considered positive.

2.6. Histological Analysis

After completion of the study, mice were killed and perfused with heparinized (50 U/mL) phosphate-buffered saline 5/100 (PBS). Then, the heart was harvested and cryomounted in optimal cutting embedding medium (CellPath, ThermoFisher Scientific, Waltham, MA, USA). For each animal, eight 10 µm thick slices were collected. Cryosections were placed in 5% silver nitrate solution for 30–60 min then fixed in 5% sodium-thiosulfate solution for 2–3 min. Sections were parallel to the valve plane and digitized using ScanScope CS (Leica Biosystems, Wetzlar, Germany). Regions of interest (ROI) encompassing the valve leaflets and the aortic sinus were manually drawn using Aperio ImageScope software v12.3 (Leica Biosystem, Wetzlar, Germany). The valvular ROIs were processed to detect the rate of von Kossa staining, using Python programing language (Python Software Foundation, www.python.org, accessed on 29 November 2018) and OpenSlide [Geospatial Data Abstraction Library (GDAL) and Mahotas] for image processing [16]. The aortic valve leaflet area, the area of aortic sinus tissue, and the percentage of von Kossa staining within the aortic valve leaflets were assessed. Results were expressed as the relative proportion of stained tissue to total tissue area.

2.7. Irradiation of Human Aortic Valvular Interstitial Cells

As the valvular interstitial cells (hVICs) play a central role in the aortic valve mineralization, we also investigated the possible negative impact of irradiation on the viability of hVICs. Human aortic tricuspid valves were collected anonymously from patients with calcific aortic valve disease undergoing valve replacement surgery at Rouen University Hospital (Rouen, France). In accordance with French legislation, the patients gave their informed consent to participation. The study was approved by the regional ethics committee (Comité de Protection des Personnes Nord Ouest I, Rouen, France, 2 May 2016) and the patients provided informed consent. The hVICs were isolated from non-calcified areas of the valves as previously described [17,18]. Gibco™ Dulbecco's modified Eagle's medium (DMEM), high glucose with Gluta-MAX™ 11574456 (Fisher Scientific™) supplemented with 10% fetal bovine serum (FBS) 11573397 (Fisher Scientific™), and 1% antibiotics (100 IU/mL penicillin-G-Na; 50 IU/mL streptomycin sulfate) was used for cell culture. Experiments were performed on cells, in T25 flasks, from passages 2 to 4. Irradiations (10 or 20 Gy) were performed using the same Pxi225CX micro-irradiator.

The cell viability of hVICs was studied by flow cytometry. Twenty-four hours after irradiation, the supernatant was collected and cells were resuspended by trypsination. Supernatant and cells were centrifuged and incubated for 10 min in PBS solution with 20 µg/mL of propidium iodide. Propidium iodide staining was analyzed by the Cytoflex-GalliosTM flow cytometer (Beckman Coulter SAS, Marseille, France). The number of viable cells in each culture after irradiation was achieved based on the CytExpert 2.4 Flow Analysis software (Beckman Coulter SAS, Marseille, France).

Twenty-four hours after irradiation protocol, the cells were washed with cold PBS, and resuspended by trypsination. Cells were fixed in 70% ethanol solution. The cell cycle of hVICs was studied by flow cytometry with a classical propidium iodide (50 µg/mL, life technologies, Carlsbad, CA, USA) solution with RNase A (20 mg/mL, life technologies) in PBS. Propidium iodide staining was analyzed by the Cytoflex-GalliosTM flow cytometer (Beckman Coulter SAS, Marseille, France). The analysis and determination of the cell distribution in each phase of the cell cycle was achieved based on the Kaluza® Flow Analysis software (Beckman Coulter SAS, Marseille, France).

2.8. Statistical Analysis

Values were expressed as mean ± SEM. A linear model analysis was used to evaluate the effect of the time, the genotype, and the radiation dose. A post hoc analysis was performed using Tukey HSD test only when the lineal model was significant. Otherwise, i.e., when the global *p*-value for the linear model was not significant, no post hoc effect was performed. A linear regression was used to correlate the quantitative analysis of von Kossa staining and sinus lesion area with peak aortic jet velocity. For proportions, the Fischer exact test was used to compare differences between groups. Statistical analyses were performed using JMP 11 (SAS Institute, Cary, NC, USA), and a p value ≤ 0.05 was considered statistically significant.

3. Results

Radiation exposure was well tolerated in all animals.

3.1. Echocardiography

In ApoE$^{-/-}$ mice, there was an impairment of left ventricular function compared to WT mice, as demonstrated by increased LVDs resulting in a decreased LV fractional shortening (see Table 1). There was no effect of radiation exposure on left ventricular function over time. On the other hand, the peak aortic jet velocity was significantly higher at the 3-month follow-up in ApoE$^{-/-}$ mice ($p < 0.0001$) compared to WT.

Table 1. Echocardiography: left ventricle dimensions at baseline and 3-month follow-up.

	Baseline		3-Month		*p*-Values			
	WT	**ApoE$^{-/-}$**	**WT**	**ApoE$^{-/-}$**	**Global *p*-Value**	**Time Effect**	**Genotype Effect**	**Radiation Effect**
IVSd (mm)	0.72 ± 0.02	0.75 ± 0.01	0.67 ± 0.02	0.68 ± 0.01	0.0009	0.0002	ns	ns
IVSs (mm)	0.89 ± 0.05	0.8 ± 0.02	0.72 ± 0.02	0.76 ± 0.03	0.01	0.0005	ns	ns
LVDd (mm)	3.70 ± 0.06	3.75 ± 0.09	3.95 ± 0.06	4.12 ± 0.09	<0.01	0.0002	ns	ns
LVDs (mm)	2.48 ± 0.07	2.59 ± 0.08	2.84 ± 0.06	3.13 ± 0.1	<0.0001	<0.0001	0.01	ns
LVPWd (mm)	0.75 ± 0.02	0.86 ± 0.04	0.82 ± 0.03	0.83 ± 0.03	ns	nd	nd	nd
LVPWs (mm)	0.98 ± 0.03	1.07 ± 0.04	0.98 ± 0.03	0.98 ± 0.04	ns	nd	nd	nd
FS (%)	33.13 ± 1.32	31.15 ± 1.08	28.27 ± 0.67	24.40 ± 1.23	<0.0001	<0.0001	<0.01	ns

Measurements were performed in 22 WT and 25 ApoE$^{-/-}$ mice. IVS: interventricular septum, LVD: left ventricle diameter, LVPW: left ventricle posterior wall, FS: fractional shortening, d: diastole, s: systole, ns, not significant, nd: not done (i.e., post hoc tests were not performed in case of a non-significant global *p*-value for the model). Data are expressed as mean ± SEM.

As shown in Figure 2 and Table 2, radiation exposure resulted in a further increase in peak aortic jet velocity at 3 months ($p < 0.001$), suggesting a radiation-induced aortic valve remodeling.

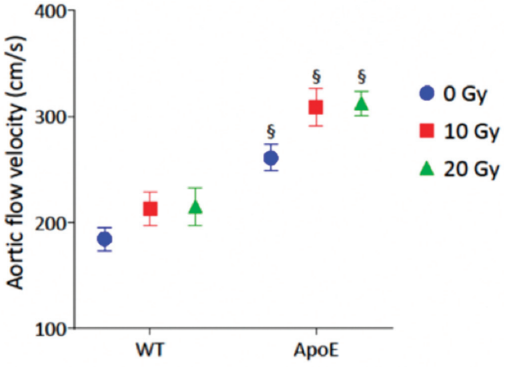

Figure 2. Impact of radiation exposure dose on peak aortic jet velocity at 3-month follow-up. The analysis was performed in WT control (n = 10), WT 10 Gy (n = 6), WT 20 Gy (n = 6), ApoE$^{-/-}$ control (n = 11), ApoE$^{-/-}$ 10 Gy (n = 4), and ApoE$^{-/-}$ 20 Gy (n = 10). Data are expressed in mean ± SEM, § $p < 0.05$ vs. dose-equivalent WT.

Table 2. Functional aortic valve assessment using echocardiography at 3-month follow-up.

	WT			ApoE$^{-/-}$			*p*-Value		
Radiation Dose	**0 Gy**	**10 Gy**	**20 Gy**	**0 Gy**	**10 Gy**	**20 Gy**	**Global *p*-Value**	**Genotype Effect**	**Radiation Effect**
Flow velocity (cm/s)	184 ± 5	213 ± 10	214 ± 2	261 ± 17 §	308 ± 20 §	312 ± 15 *§	<0.0001	<0.0001	<0.001
Mean gradient (mmHg)	6.33 ± 0.46	8.53 ± 0.93	8.83 ± 0.44	13.58 ± 1.69 §	16.92 ± 1.71 §	20.57 ± 2.04 §*	<0.0001	<0.0001	<0.01
Max gradient (mmHg)	13.68 ± 0.78	18.36 ± 1.88	18.45 ± 0.4	28.05 ± 3.53 §	38.57 ± 5.02 §	39.75 ± 3.84 §	<0.0001	<0.0001	<0.01

Peak aortic jet flow velocity, mean, and maximal trans-valvular gradients were assessed using pulse wave Doppler recordings. Data are expressed in mean ± SEM, * $p < 0.05$ vs. ApoE$^{-/-}$ and § $p < 0.05$ vs. WT with equivalent radiation dose.

3.2. α-VCAM MPIO MR Imaging Findings

Twenty-three animals (WT: n = 9, ApoE$^{-/-}$: n = 14) underwent α-VCAM MPIO MR imaging. A T2* signal void was noted in the aortic sinus in 15/23 (65%) cases and in the aortic valve leaflets in 18/23 (78%) cases (Table 3).

Table 3. Proportion of MPIO-αVCAM-1 binding using MR imaging in aortic valve leaflets and aortic sinus.

	Aortic Sinus			Aortic Valve Leaflets		
MPIO-αVCAM-1	**Negative**	**Positive**	**Total**	**Negative**	**Positive**	**Total**
RT-	4	2	6	4	2	6
RT+	4	13	17	1	16	17
Total	8	15	23	5	18	23

Figure 3 depicts a T2* signal void involving both the aortic sinus and valve leaflets in an ApoE$^{-/-}$ mice imaged 3 months after irradiation. The association of the T2* signal void with radiation exposure reached statistical significance within the aortic valve leaflets (Fischer exact test $p < 0.01$), but not within the aortic sinus, suggesting a specific impact of targeted irradiation on the expression of VCAM-1 within the aortic valve endothelium.

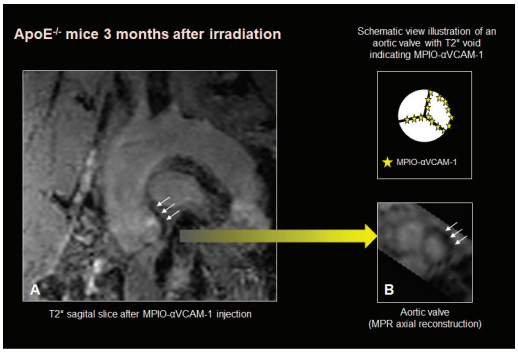

Figure 3. Example of a T2* signal void involving both the aortic sinus and the valve leaflets in an ApoE$^{-/-}$ mice imaged 3 months after irradiation. (white arrows indicating the signal void). (**A**): sagittal view, (**B**): short axis reconstruction of the aortic valve with the corresponding scheme depicting the MPIO-αVCAM–1 (yellow stars) on the valve leaflets.

3.3. Histological Analysis

Histological findings showed that, independently of radiation exposure, ApoE$^{-/-}$ mice showed an aortic sinus thickening demonstrated by an increased aortic sinus tissue area compared to WT (Table 4).

Table 4. Impact of radiation exposure on histological findings.

	WT			ApoE$^{-/-}$			*p*-Values		
	0 Gy	**10 Gy**	**20 Gy**	**0 Gy**	**10 Gy**	**20 Gy**	**Global**	**Genotype**	**Radiation**
Leaflet area (mm^2)	0.144 ± 0.006	0.161 ± 0.015	0.166 ± 0.015	0.115 ± 0.005	0.164 ± 0.008 *	0.195 ± 0.019 *	0.0001	ns	0.0001
Sinus tissue area (mm^2)	0.253 ± 0.010	0.245 ± 0.009	0.217 ± 0.022	0.473 ± 0.030 §	0.484 ± 0.026 §	0.697 ± 0.034 *†§	<0.0001	<0.0001	<0.05
von Kossa area (%)	4.187 ± 11.7	3.809 ± 4.4	5.224 ± 1.62	4.224 ± 6.76	7.027 ± 10.287 *	4.027 ± 4.584 *†	<0.0001	ns	<0.0001

Mineralization was assessed using the percentage of von Kossa staining in aortic valve leaflets in 4 WT RT-, 3 WT 10 Gy, 3 WT 20 Gy, 4 ApoE$^{-/-}$ RT-, 4 ApoE$^{-/-}$ 10 Gy, and 3 ApoE$^{-/-}$ 20 Gy. Data are expressed as mean ± SEM, * $p < 0.05$ vs. ApoE$^{-/-}$ RT-, † $p < 0.05$ vs. ApoE$^{-/-}$ 10 Gy, § $p < 0.05$ vs. dose-equivalent WT. ns, not significant.

In addition, there was a significant remodeling of valve leaflets and aortic sinus wall related to radiation exposure, as demonstrated by the increased tissue area, especially in ApoE$^{-/-}$ mice. This remodeling further increased with the radiation dose in ApoE$^{-/-}$. Von Kossa staining showed that radiation exposure promoted a mineralization process in the valve leaflets ($p < 0.001$), especially in ApoE$^{-/-}$ mice. It is worth noting that the mineralization process was decreased in ApoE$^{-/-}$ after 20 Gy compared to 10 Gy. As described in Figure 4, there was a significant correlation of von Kossa staining with peak aortic jet velocity (r = 0.57, $p < 0.01$) and mean trans-valvular gradient (r = 0.55, $p = 0.02$).

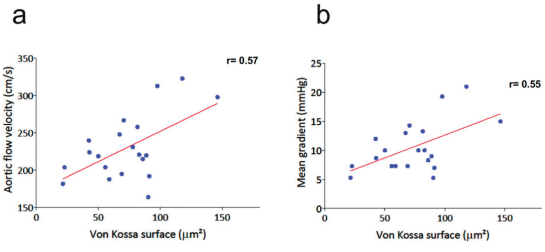

Figure 4. Correlation of von Kossa staining with peak aortic jet velocity (**a**) and mean trans-valvular gradient (**b**) in the whole study population.

3.4. hVICs Analysis

Cell cycle and viability analyses of hVICs were performed in, respectively, four and five experiments under control conditions, and 10 Gy and 20 Gy under irradiation conditions. Radiation exposure did not reduce the viability of hVICs, and had no impact on cell cycle analysis (Figure 5).

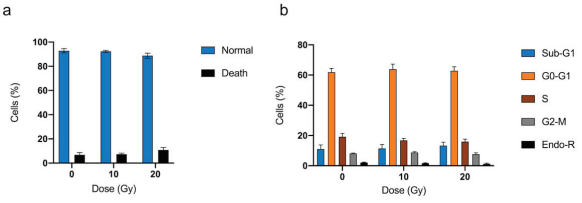

Figure 5. Impact of radiation exposure on isolated valve interstitial cells from patients using viability (**a**) and cell cycle analysis (**b**). Sub-G1: Sub-growth-phase-1; G0-G1: growth 0 and growth 1 phase; S: synthesis phase; G2-M: growth 2 phase and mitosis; Endo-R: Endo-replication. Data are expressed in mean ± SEM.

4. Discussion

The main result of this study is that in ApoE$^{-/-}$ mice, a targeted aortic valve irradiation resulted in an aortic valve remodeling demonstrated by a significant increase in peak aortic jet velocity at a 3-month follow-up, an effect that was not significant in wild type mice.

Degenerative calcified AS is characterized by fibro-calcific remodeling of the valve leaflets. The progression of the disease involves severe calcification within the valve leaflets, leading to an impairment of valve motion contributing to blood flow obstruction [11]. A high incidence of valvular dysfunction has been reported in populations undergoing mediastinal radiation therapy [19]. In these patients, the progression of a pre-existing AS is accelerated, underlining the potential of X-rays to initiate and develop valvular lesions [4]. Previous results in patients requiring valve surgery described specific features of radiation-induced valve lesions, including various levels of diffuse leaflet fibrosis and retractions, which differentiate these radiation-induced lesions from degenerative calcified valvular stenosis [20].

In vitro studies emphasized the relationship between irradiation and the behavior of hVICs, which are the main cell type in aortic valve cusps. Early results showed that a 10 Gy irradiation of hVICs induced an osteogenic phenotype differentiation demonstrated by a significant increase in bone morphogenetic protein 2, osteopontin, alkaline phosphatase, and Runx2 [10]. In addition, low-dose-radiation exposure of porcine valvular interstitial cells resulted in myfibroblast-like changes associated with calcification, while high doses equivalent to 60 Gy over 30 fractions produced DNA damage leading to a decrease in cell viability [21]. In the present study, we found no reduction in cell viability and no impact on cell cycle in hVICs exposed to 10 Gy or to 20 Gy. Although valve remodeling increased with the radiation dose, as demonstrated by the increased amount of tissue within the aortic sinus, increasing radiation from 10 to 20 Gy in ApoE$^{-/-}$ mice did not result in a further increase in aortic peak velocity. This is in agreement with previous findings by Meerman et al. [21], who found that calcification, assessed by alkaline phosphatase activity, was mostly present in VICs exposed to 4 Gy, while higher doses equivalent to 60 Gy resulted in giant fibroblast-like cell changes. Our findings are in agreement with these results, suggesting that low-dose irradiation (up to 20 Gy) may induce an osteogenic transition without cell death. This is also in agreement with clinical findings demonstrating a mix of fibrosis and calcifications in patients with a history of mediastinal radiation therapy [20].

In a recent study, Mpweme et al. [9] demonstrated that radiation-induced aortic valve remodeling was inhibited in TRPM4$^{-/-}$ mice. TRPM4 is a monovalent non-selective cation channel involved in calcium handling and participating in fibroblast transition to myofibroblasts, a phenomenon observed during aortic valve stenosis. In their study, maxi-

mal aortic valve jet velocity was evaluated at a 5-month follow-up and was significantly higher in irradiated compared to non-irradiated wild type Trpm4$^{+/+}$ mice (240.9 ± 17.2 and 185.1 ± 7.9 cm·s^{-1}, respectively), while no difference was observed in Trpm4$^{-/-}$ animals depending on irradiation. The authors also noted that irradiation induced a significant increase in cusp surface in Trpm4$^{+/+}$ mice compared to Trpm4$^{-/-}$ animals, as well as in the total surface of the cusp and aortic annulus, with a linear correlation between these pathological findings and maximal aortic jet velocity. Compared to this latter study, we found a slightly lower aortic jet velocity at the 3-month follow-up after radiation exposure in WT animals, whereas it was further increased in ApoE$^{-/-}$. This aortic valve remodeling in ApoE$^{-/-}$ mice was associated with valve mineralization that was not observed in WT mice, in agreement with previous findings from Mpweme et al. [9]. In addition, there was a significant relationship between von Kossa staining and peak aortic jet velocity. These results demonstrated that, compared to WT animals, ApoE$^{-/-}$ mice are more likely to develop accelerated aortic lesions after irradiation, as demonstrated by ultrasound and histological findings.

In humans, the risk of radiation-induced peripheral atherosclerosis is significantly increased when cardiovascular risk factors are combined with radiation therapy [12]. Although there is a lack of research documenting the impact of combined risk factors and radiation on the occurrence of aortic stenosis, it has been demonstrated that pre-existing risk factors are strongly associated with severe calcific AS [22]. Similarly, calcific deposition has been previously reported in a mouse model of high-fat-diet-induced AS [23,24]. In addition, valve leaflet thickening was also found in Ldlr$^{-/-}$ Apob100/100 mice with a 0.15% cholesterol diet, associated with an increase in peak aortic jet velocity which was rescued by a regular exercise training [25]. To our knowledge, our study is the first preclinical investigation evaluating the impact of targeted radiation exposure on the development of AS in ApoE$^{-/-}$ mice. Previous results showed that C57BL/6J mice fed with a Western diet may demonstrate inflammatory features similar to early atherosclerotic lesions [26]. ApoE$^{-/-}$ mice of C57BL/6 background develop atherosclerosis throughout the arterial tree, including the aortic root at the base of the valve, and these lesions, a condition that favors vascular inflammation, are accelerated when mice are fed with a Western diet [13]. Using echocardiography and histologic examination, Tanaka et al. [14] documented sclerotic changes associated with functional abnormalities in senile ApoE$^{-/-}$ mice yielding aortic valve sclerosis that was similar to the results we observed in younger individuals after targeted aortic valve irradiation. These results are in line with human investigations demonstrating an acceleration of aortic valve lesions after thoracic irradiation [20].

As irradiation in itself induces local inflammation, it is likely that it is synergistic with the inflammatory features of ApoE$^{-/-}$ mice and further accelerates the remodeling process. In this study, aortic lesions were associated with persistent inflammation, as demonstrated by non-invasive MPIO-αVCAM1 MR imaging. Previous studies demonstrated that VCAM-MPIO binding, evidenced as signal voids in T2* MR images, correlated well with endothelial VCAM-1 upregulation. These results were observed in different experimental settings, including the tumor–brain interface [27], systemic inflammation [15], and atherosclerosis [28]. We observed a signal void limited to the aortic valve and annulus, i.e., the irradiation target, confirming the usefulness of a targeted irradiation to the aortic valve using the MRI-based anatomic atlas. However, despite a strong association between MR findings and aortic valve irradiation, some animals presented a T2* signal void although they received no radiation, whereas some irradiated mice presented a normal MR signal. This is compatible with previous findings demonstrating early spontaneous vascular inflammation in C57BL6/J and ApoE$^{-/-}$ mice strains. Especially, all phases of atherogenesis have been demonstrated in ApoE$^{-/-}$ mice, from the early inflammatory response with monocyte adhesion to late fibrous caps [13]. Consequently, it is likely that the relative heterogeneity of T2* MR results reflects the temporal heterogeneity of the inflammatory process accelerated by radiation exposure.

As late development of radiation-induced aortic stenosis remains a clinical issue in patients receiving thoracic radiation therapy, this preclinical model could be useful in assessing factors that may either accelerate, like a high-fat diet, a condition associated with aortic valve remodeling, or inhibit the pathological process, like lipid-lowering therapies or inflammation modulation [29]. For example, the model might be appropriate to evaluate the involvement of NF-κB signaling, which is a mediator of various inflammatory processes involved in carcinogenesis, radiation-induced inflammation, and in the pathogenesis of various cardiovascular diseases [30]. Recently, Candellier et al. demonstrated that indoxyl-sulfate promotes osteogenic differentiation of hVIC via an activation of the AhR-NF-κB pathway [29]. Our preclinical model could be used to evaluate the impact of therapies inhibiting NF-κB signaling, including melatonin [31], aspirin [32], or metformin [33], on radiation-induced aortic remodeling.

Limitations

The phenotypic changes of valve interstitial cells into osteoblast-like cells were not evaluated in this study. Due to the very tiny size of the aortic valve in mice, it appeared difficult to obtain a sufficient amount of valve tissue to assess the production of osteogenic factors such as osteopontin, alkaline phosphatase, or the transcription factor Runx2 using blotting techniques. However, this phenomenon was previously demonstrated by Nadlonek et al. [10] using hVICs isolated from normal aortic valves and exposed to 10 Gy irradiation.

It remains complicated to compare the dose radiation of radiation exposure in mice with the doses administrated to humans. The murine response to irradiation varies from human cellular and molecular pathways, and the complexity of radiation treatment in humans is hardly reproducible using preclinical protocols. In addition, there is no clear consensus on the radiation dose, dose rate, and fractionation that should be used in murine models of cardiac radiation toxicity [34]. The dose and fractionation chosen in this study is compatible with previous studies in the field and ensured a good tolerance of radiation exposure in mice. In clinical practice, radiation therapy is often administrated in multiple smaller radiation fractions. As the risk of radiation-induced atherosclerosis is influenced by dose fractionation [35], the effect of hypofractionation of irradiation in this animal model of aortic valve radiotoxicity remains to be investigated.

5. Conclusions

This study demonstrated that targeted aortic valve irradiation in ApoE$^{-/-}$ mice resulted in the development of an aortic valve remodeling that mimics the human radiation-induced aortic valve stenosis, with a higher effect than in WT animals. This novel animal model could be useful for the preclinical assessment of therapies that may affect delayed aortic valve disease after irradiation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm12185854/s1.

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Institutional Review Board Statement: All animal experiments were approved by the institutional animal ethics committee (Cenomexa #12314 and #18217) in line with EU Directive 2010/63/EU for animal experiments and the ARRIVE guidelines, and authorized by the French Ministry of Higher

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data supporting this study will be made available to qualified researchers upon reasonable request from the corresponding author.

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Predictors of Conduction Disturbances Requiring New Permanent Pacemaker Implantation following Transcatheter Aortic Valve Implantation Using the Evolut Series

Mahmoud Abdelshafy [1,2,3], Ahmed Elkoumy [1,2,4], Hesham Elzomor [1,2,4], Mohammad Abdelghani [3,5], Ruth Campbell [1], Ciara Kennedy [1], William Kenny Gibson [1], Simone Fezzi [1], Philip Nolan [1], Max Wagener [1], Shahram Arsang-Jang [2,6], Sameh K. Mohamed [2], Mansour Mostafa [3], Islam Shawky [3], Briain MacNeill [1], Angela McInerney [1], Darren Mylotte [1,6,*] and Osama Soliman [1,2,7,*]

1 Discipline of Cardiology, Galway University Hospital, SAOLTA Healthcare Group, Health Service Executive, H91 YR71 Galway, Ireland; mahmoud.abdelshafy@universityofgalway.ie (M.A.); aelkoumy@universityofgalway.ie (A.E.); hesham.elzomor@universityofgalway.ie (H.E.); missruthc@gmail.com (R.C.); c.kennedy40@nuigalway.ie (C.K.); billykgibson@gmail.com (W.K.G.); fezzisimone@gmail.com (S.F.); philipnolan92@gmail.com (P.N.); max.wagener@gmail.com (M.W.); briain.macneill@icloud.com (B.M.); angela_mcinerney@hotmail.com (A.M.)
2 CORRIB Core Lab, University of Galway, H91 V4AY Galway, Ireland; shahram.arsang-jang@universityofgalway.ie (S.A.-J.); sameh.kamaleldin@gmail.com (S.K.M.)
3 Department of Cardiology, Al-Azhar University, Cairo 11311, Egypt; m.abdelghani.nl@gmail.com (M.A.); mans_aref@yahoo.com (M.M.); islam012@hotmail.com (I.S.)
4 Islamic Center of Cardiology and Cardiac Surgery, Al-Azhar University, Cairo 11651, Egypt
5 Department of Cardiology, Amsterdam UMC, Amsterdam Cardiovascular Sciences, University of Amsterdam, 1081 HV Amsterdam, The Netherlands
6 Discipline of Medicine, Clinical Science Institute, University of Galway, H91 YR71 Galway, Ireland
7 CÚRAM Centre for Medical Devices, H91 TK33 Galway, Ireland
* Correspondence: darrenmylotte@gmail.com (D.M.); o.i.soliman@gmail.com (O.S.)

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Abstract: (1) Background: Conduction disturbance requiring a new permanent pacemaker (PPM) after transcatheter aortic valve implantation (TAVI) has traditionally been a common complication. New implantation techniques with self-expanding platforms have reportedly reduced the incidence of PPM. We sought to investigate the predictors of PPM at 30 days after TAVI using Evolut R/PRO/PRO+; (2) Methods: Consecutive patients who underwent TAVI with the Evolut platform between October 2019 and August 2022 at University Hospital Galway, Ireland, were included. Patients who had a prior PPM (n = 10), valve-in-valve procedures (n = 8) or received >1 valve during the index procedure (n = 3) were excluded. Baseline clinical, electrocardiographic (ECG), echocardiographic and multislice computed tomography (MSCT) parameters were analyzed. Pre-TAVI MSCT analysis included membranous septum (MS) length, a semi-quantitative calcification analysis of the aortic valve leaflets, left ventricular outflow tract, and mitral annulus. Furthermore, the implantation depth (ID) was measured from the final aortography. Multivariate binary logistic analysis and receiver operating characteristic (ROC) curve analysis were used to identify independent predictors and the optimal MS and ID cutoff values to predict new PPM requirements, respectively; (3) Results: A total of 129 TAVI patients were included (age = 81.3 ± 5.3 years; 36% female; median EuroSCORE II 3.2 [2.0, 5.4]). Fifteen patients (11.6%) required PPM after 30 days. The patients requiring new PPM at 30 days were more likely to have a lower European System for Cardiac Operative Risk Evaluation II, increased prevalence of right bundle branch block (RBBB) at baseline ECG, have a higher mitral annular calcification severity and have a shorter MS on preprocedural MSCT analysis, and have a ID, as shown on the final aortogram. From the multivariate analysis, pre-TAVI RBBB, MS length, and ID were shown to be predictors of new PPM. An MS length of <2.85 mm (AUC = 0.85, 95%CI: (0.77, 0.93)) and ID of >3.99 mm (area under the curve (AUC) = 0.79, (95% confidence interval (CI): (0.68, 0.90)) were found to be the optimal cut-offs for predicting new PPM requirements; (4) Conclusions: Membranous septum length and implantation depth were found to be independent predictors of new PPM post-TAVI with the Evolut platform. Patient-specific implantation depth could be used to mitigate the requirement for new PPM.

Keywords: TAVI; conduction disturbance; computed tomography; pacemaker implantation; membranous septum

1. Introduction

Transcatheter aortic valve implantation (TAVI) is an established treatment option for older patients with symptomatic severe aortic stenosis, irrespective of operative risk [1,2]. New conduction disturbances, particularly new left bundle branch block (LBBB) and permanent pacemaker (PPM) implantation, have been associated with increased all-cause mortality and heart failure hospitalization at one year [3]. The incidence of conduction disturbance and the need for new PPM remains frequent, despite the advancements in device technology and implantation techniques [4]. The direct compression of conduction tissue by the transcatheter heart valve (THV), resulting in local ischemia, oedema, and haemorrhage, may explain the injury of the often calcific conductive system [5].

The rate of new PPM was five times more frequent with the self-expanding first-generation CoreValve system (Medtronic, Dublin, Ireland) (25–28%) compared with balloon-expanding valves (5–7%) (SAPIEN and SAPIEN XT; Edwards Lifesciences, Irvine, CA, USA) [6,7]. Recent studies have shown that the PPM rates obtained using the SAPIEN 3 and SAPIEN 3 Ultra valves can be as low as 4.4–6.5% [2,8]. The introduction of newer-generation CoreValve systems (Evolut R/Pro/Pro+) with novel features, such as the ability to recapture and reposition, has been associated with a lower rate of new PPM [1]. When combined with increasing operator experience and novel imaging and implantation techniques, the rate of new PPM with contemporary self-expanding platforms is lower, but the data are less robust, falling short of a continuous technology/technique dynamicity.

Previously identified predictors of new PPM post-TAVI are older age, right bundle branch block (RBBB) on baseline electrocardiogram (ECG), higher mean aortic valve gradient, calcification including left ventricular outflow tract (LVOT) or mitral annulus, membranous septum (MS) length and implantation depth (ID) [4,9]. Multislice computed tomography (MSCT) can identify the MS, which serves as an anatomical landmark and represents the distance between the aortic annulus and the atrioventricular conduction system. The ID plays a vital role as a modifiable predictor of new PPM and is the focus of ongoing investigations on TAVI using the Evolut platform. It appears that implanting the device higher in relation to the length of the MS can reduce the likelihood of post-TAVI PPM risk [10–13].

In this study, we aimed to investigate the rate and predictors of conduction disturbance requiring a new PPM after TAVI with the Evolut R/PRO/PRO+ systems in contemporary clinical practice.

2. Materials and Methods

2.1. Study Design and Patient Population

This is a single-centre, retrospective, observational study. Consecutive patients who underwent TAVI with the Medtronic Evolut R/PRO/PRO+ system between October 2019 and August 2022 at University Hospital Galway, Ireland, were reviewed for inclusion eligibility. Patients with prior PPM undergoing a valve-in-valve procedure, or patients who received >1 valve during the index procedure, were excluded. The study complied with the ethical guidelines of the Declaration of Helsinki and was approved by the institutional ethical committee.

2.2. TAVI Procedure

Pre-procedure workup included baseline ECG, transthoracic echocardiography (TTE), and multislice computed tomography (MSCT). The heart team determined eligibility for TAVI in all cases. Standard in-hospital care post-TAVI included daily ECG until hospital discharge. Echocardiography was performed in all cases post-TAVI. TAVI procedure

was performed in accordance with the instructions for use and the hospital's standard procedure. Valve release was performed under fast or rapid pacing, with an optimal final ID of 3–5 mm. Local anaesthesia was used, except in exceptional circumstances when general anaesthesia was used. Pre- and/or post dilatation was performed at the discretion of the operating team.

2.3. MSCT Analysis

The pre-TAVI MSCT was analysed according to the recommendations of the Society of Cardiovascular Computed Tomography [14]. The reconstruction and analysis were performed using 3mensio Structural Heart software program version 10.3 (Pie Medical Imaging, Maastricht, The Netherlands). Calcification of the valvular apparatus at aortic cusps and left ventricular outflow tract was visually graded as none = 0, mild = 1, moderate = 2, and severe = 3. The index of annular eccentricity was calculated as and the degree of oversizing by area as [(prosthesis area/annulus area $-$ 1) $\times$ 100%] and by perimeter as [(prosthesis perimeter/annulus perimeter $-$ 1) $\times$ 100%]. Mitral annular calcification (MAC) was defined as the presence of dense calcium deposits at the base of mitral leaflets, grade 0 = no MAC, grade 1 = mild MAC affecting $\leq$ 25% of the annulus, grade 2 = moderate MAC affecting 25–50% of the annulus, grade 3 = severe MAC affecting $\geq$50% of the annulus [15]. The MS length measurement was performed by an independent imaging cardiologist blinded to post-TAVI outcomes. For a standardized analysis, the cursor in the perpendicular co-planar view was placed at the intersection of the non-coronary and right coronary cusp. MS was defined on this perpendicular co-planar view as the thinnest part of the interventricular septum between LVOT and the right atrium from the nadir of the non-coronary cusp to the tip of the muscular interventricular septum [12,13].

2.4. ID Measurement

The ID was determined on the final aortogram post-TAVI and was measured as the depth from the edge of the THV frame up to the nadir of the non-coronary cusp (NCC) [12].

2.5. ECG Data

A 12-lead ECG was collected at three timepoints: baseline (within 24 h before the procedure), immediately after the procedure (post-TAVI), and at hospital discharge. The diagnosis of conduction abnormalities was classified according to the recommendations of the American Heart Association/American College of Cardiology Foundation/Heart Rhythm Society (AHA/ACCF/HRS) for the standardization and interpretation of ECGs [16]. PR interval and QRS duration were analysed for each ECG to calculate the change (delta) from baseline to post-TAVR and the change from baseline to discharge.

2.6. Clinical Data and TAVI Clinical Outcome

The clinical data were obtained from a prospectively managed, dedicated database within Galway University Hospital. Clinical outcomes were defined based on the Valve Academic Research Consortium-3 (VARC-3) consensus document [17].

2.7. Study Outcome

The primary outcome of our study was to investigate the predictors of new PPM post-Evolut implantation at 30 days, while the secondary outcome was to explore the changes in the PR interval and QRS duration from baseline to post-TAVI and pre-discharge.

2.8. Statistical Analysis

Categorical variables were presented as numbers and percentages. Continuous variables were reported as mean $\pm$ standard deviation (SD) or median and interquartile range (IQR) as appropriate. The Shapiro–Wilk test was used to test the normality of continuous variables. Baseline patient characteristics, comorbidities, ECG data, echocardiographic data, MSCT data, procedural and post-procedural parameters were compared between

those requiring and not requiring a new PPM. Continuous data were compared using Student's *t*-test (normality) or Mann–Whitney U test (non-normality). Categorical data were compared using chi-square test or Fisher's exact test. Independent predictors of new PPM were determined using binary logistic regression and the backward method for variable selection. Odds ratios (ORs), along with their corresponding 95% confidence intervals (CIs), were used to report the results. The variables included in the univariable analysis were the European System for Cardiac Operative Risk Evaluation II (EuroSCORE II), right bundle branch block (RBBB), membranous septum (MS) length, more than/equal moderate mitral annular calcification (MAC), implantation depth (ID) and the difference between the MS length and the ID. Parameters with a p-value ≤ 0.01 in univariate analyses were included in multivariate analyses. The variables included in the multivariable analysis were RBBB, MS length and ID. A p-value of less than 0.05 in multivariate analysis was considered statistically significant. Receiver operating characteristic (ROC) curve analysis was employed to identify the preprocedural and procedural parameters that best predict new PPM and to determine the optimal cut-off value for that/those parameter(s). All statistical analyses were performed using SPSS Statistics for Windows, version 25.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

Between April 2019 and August 2022, 150 patients were treated with the Medtronic Evolut platform. After the exclusion of patients who had PPM at baseline (n = 10), patients who had a valve-in-valve procedure (n = 8), and patients who received >1 valve during the index procedure (n = 3), the final cohort included 129 patients (Figure 1).

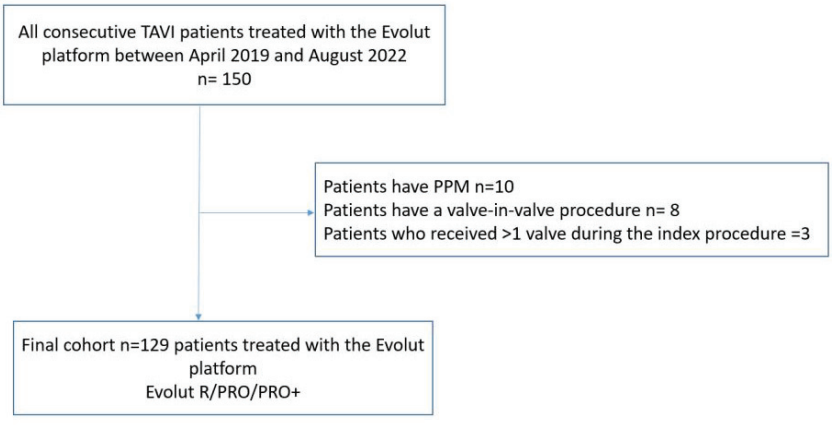

Figure 1. Study Flow chart.

3.1. Baseline Characteristics

The average age was 81.3 (±5.3) years, and one-third (36%) were female. The median European System for Cardiac Operative Risk Evaluation (EuroSCORE) II was 3.2 [2.0, 5.4]. All underwent transfemoral TAVI with local anaesthesia, except for two patients with general anaesthesia. Balloon pre-dilation was used in 69%, and balloon post-dilation was performed in 40%. The measurement of implantation depth was only feasible in 106 patients. The baseline demographic, clinical, ECG Echocardiographic and MSCT characteristics are detailed in Table 1, and procedural variables post-TAVI complications are displayed in Table 2.

Table 1. Baseline demographic, clinical, ECG, echocardiographic and MSCT characteristics.

	All Patients $n = 129$	PPM $n = 15$	No PPM $n = 114$	p Value
Baseline characteristics				
Age (years)	81.3 ± 5.3	81.7 ± 4.3	82.1 ± 5.3	0.36
Female, *n* (%)	46 (36%)	2 (13%)	44 (38.6%)	0.08
Body Mass Index (kg/m^2)	26.8 [24.1, 31.1]	30 [23.7, 33.8]	26.8 [24.1, 30.7]	0.35
Hypertension, *n* (%)	99 (77%)	13 (87%)	86 (75%)	0.51
Diabetes, *n* (%)	43 (33%)	7 (47%)	36 (32%)	0.25
Dyslipidemia, *n* (%)	73 (57%)	12 (80%)	61 (54%)	0.058
NYHA class ≥ III, *n* (%)	76 (59%)	6 (40%)	70 (61.4%)	0.16
COPD, *n* (%)	22 (17%)	2 (13%)	20 (18%)	>0.999
Previous MI, *n* (%)	19 (15%)	2 (13%)	17 (15%)	>0.999
Prior CVA	14 (11%)	4 (27%)	10 (9%)	0.059
Glomerular filtration rate (mL/min/1.73 m^2)	61.1 ± 16.7	59.1 ± 15.4	55.6 ± 20.3	0.42
EuroSCORE II	3.2 [2.0, 5.4]	1.9 [1.7, 3.2]	3.3 [2.1, 5.4]	0.008
Baseline ECG				
Atrial Fibrillation, *n* (%)	28 (22%)	2 (13%)	26 (23%)	0.52
RBBB	16 (12%)	7 (47%)	9 (8%)	<0.001
LBBB	12 (10%)	0 (0%)	12 (11%)	0.35
1st-degree AV block	32 (25%)	4 (27%)	28 (25%)	0.92
PR interval	183 [164, 209.5]	178 [156, 207.5]	184 [164, 212]	0.57
QRS duration	101 [89, 119]	120 [89, 140]	101 [89, 113]	0.18
Echocardiographic data				
LVEF ≤ 40%	33 (26%)	3 (20%)	30 (26%)	0.75
Mean AoV gradient (mmHg)	54.1 ± 32.4	60.1 ± 15.7	53.3 ± 34.1	0.45
Peak AoV gradient (mmHg)	80.8 ± 20.5	89.1 ± 18.3	79.6 ± 20.7	0.45
MSCT characteristics				
Bicuspid morphology	26 (20%)	5 (33%)	21 (18%)	0.18
Annulus diameter (mm)	25.3± 2.5	26.3± 2.7	25.2 ± 2.4	0.08
Annular eccentricity index	0.25 ± 0.06	0.27 ± 0.05	0.24 ± 0.06	0.17
Perimeter-derived annulus diameter (mm)	25.5 ± 2.2	26.5 ± 2.6	25.3 ± 2.3	0.09
Area-derived annulus diameter (mm)	24.9 ± 2.2	25.9 ± 2.7	24.8 ± 2.3	0.07
Annulus perimeter (mm)	80.4 ± 7.1	82.9 ± 8.1	79.9 ± 7.5	0.14
Annulus area (mm^2)	493.1 ± 88.1	530.5 ± 104.9	487.9 ± 90.9	0.09
LCA height (mm)	16.3 ± 3.3	16.1 ± 4.2	16 ± 3.2	0.93

Table 1. *Cont.*

	All Patients $n = 129$	PPM $n = 15$	No PPM $n = 114$	p Value
RCA height (mm)	18.9 ± 3.5	18.8 ± 3.8	18.7 ± 3.6	0.88
Aortic root angulation ≥ 49	56 (43%)	8 (53%)	48 (42%)	0.42
Membranous septum length (mm)	3 [2.1, 3.8]	1.5 [1.1, 2.5]	3.1 [2.3, 4]	<0.001
AoV calcification ≥ moderate	106 (82%)	14 (93%)	92 (81%)	0.30
LVOT calcification ≥ moderate	31 (24%)	4 (27%)	27 (24%)	0.75
MAC ≥ moderate	46 (36%)	9 (60%)	37 (33%)	0.047

Data presented as frequency and (percentage), mean ± standard deviation or median [interquartile range]. Abbreviations: AoV = aortic valve; AV block = atrioventricular block; COPD = chronic obstructive airway disease; CVA = cerebrovascular accident; EuroSCORE II = European System for Cardiac Operative Risk Evaluation II; LBBB = left bundle branch block; LCA = left coronary artery; LVEF = left ventricular ejection fraction; LVOT = left ventricular outflow tract; MAC = mitral annular calcification; MI = myocardial infraction; NYHA = New York Heart Association; PPM = permanent pacemaker; RBBB = right bundle branch block; RCA = right coronary artery.

Table 2. Procedural characteristics and in-hospital complications.

	All Patients $n = 129$	PPM $n = 15$	No PPM $n = 114$	p Value
Procedural characteristics				
THV type				
Evolut R	34 (26%)	5 (33%)	29 (25%)	0.78
Evolut PRO	45 (35%)	5 (33%)	40 (35%)	
Evolut PRO+	50 (39%)	5 (33%)	45 (40%)	
THV size				
23 mm	2 (2%)	0 (0%)	2 (2%)	0.81
26 mm	23 (18%)	2 (13%)	21 (18%)	
29 mm	64 (50%)	7 (47%)	57 (50%)	
34 mm	40 (31%)	6 (40%)	34 (30%)	
Oversizing by annulus perimeter	17.8 [13.6, 21.9]	18 [10.6, 20.4]	17.7 [13.9, 22]	0.39
Oversizing by annulus area	45.2 [35.1, 57]	45.9 [28.2, 55.8]	44.11 [36, 57.1]	0.35
Balloon pre-dilation *n* (%)	89 (69%)	12 (80%)	77 (68%)	0.39
Capture–redeployment attempts *n* (%)	55 (42.6%)	6 (40%)	49 (43%)	0.83
Capture–redeployment numbers	2.3 ± 1.5	2.3 ± 1.5	2.3 ± 1	0.96
Balloon post-dilation	51 (40%)	8 (53%)	43 (39%)	0.27
Cusp overlap	83 (64%)	76 (67%)	7 (47%)	0.16
Implantation depth at NCC (mm)	3.8 [2.8, 4.3]	4.4 [4.1, 5.7]	3.6 [2.6, 4.1]	<0.001
MS length minus implant depth, (mm)	−0.6 ± 2.5	−3.9 ± 1.5	−0.3 ± 2.4	<0.001
ID > MS	64 (50%)	13 (87%)	51 (45%)	0.002

Table 2. *Cont.*

	All Patients $n = 129$	PPM $n = 15$	No PPM $n = 114$	p Value
In-hospital complications				
In-hospital death	3 (2%)	0 (0%)	3 (3%)	>0.999
Periprocedural MI	0 (0%)	0 (0%)	0 (0%)	-
In-hospital stroke	5 (4%)	1 (7%)	4 (4%)	0.48
Vascular complications				
Major	0 (0%)	0 (0%)	0 (0%)	-
Minor	20 (16%)	2 (13%)	18 (16%)	>0.999
PVL ≥ moderate (echo)	8 (6%)	1 (7%)	7 (6%)	>0.999

Abbreviations: ID = implantation depth; MI = myocardial infraction; MS = membranous septum; NCC = non-coronary cusp; PVL = para-valvular leakage; THV = transcatheter heart valve.

3.2. Conduction Disturbance

The rate of new PPM was 10% (13/129) at discharge and 11.6% (15/129) at 30 days, which was unchanged at one year. Seven of these fifteen patients (47%) had a pre-existing RBBB. All PPMs were inserted due to complete heart block, except for one patient with new LBBB (QRS duration = 179 millisecond (msec)) and first-degree AV block (PR duration = 330 msec). The median time until new PPM was 2 days [1, 3.5], as detailed in Figure 2.

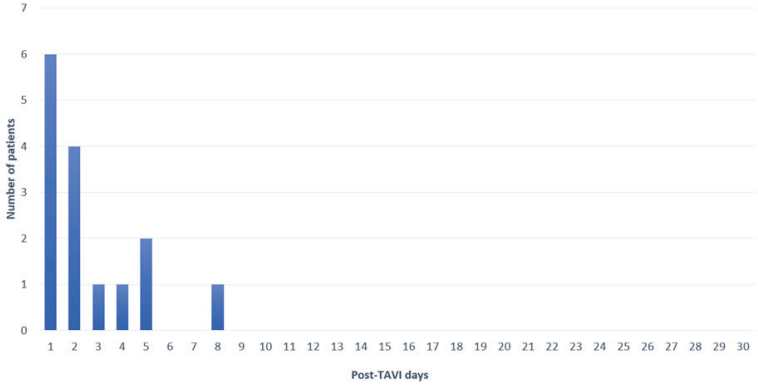

Figure 2. Time to new PPM implantation.

3.3. Predictors of New PPM

The baseline clinical characteristics of patients with and without new PPM were similar, except for EuroSCORE II, which was lower in patients with new PPM (1.9 [1.7, 3.2] vs. 3.3 [2.1, 5.4], $p = 0.008$). Patients with new PPM were also more likely to have RBBB (47% vs. 8%, $p < 0.001$), shorter MS length (1.5 [1.1, 2.5] vs. 3.1 [2.3, 4], $p = 0.002$), a higher rate of ≥moderate MAC (60% vs. 33%, $p = 0.047$) and a deeper ID (4.4 [4.1, 5.7] vs. 3.6 [2.6, 4.1], $p < 0.001$). Moreover, the difference between the MS length and the ID was significantly greater in patients who required PPM (-3.9 ± 1.5 vs. -0.3 ± 2.4, $p < 0.001$).

3.4. Multivariate Predictors of New PPM

In the multivariate model, pre-existing RBBB, MS length, and implant depth, were independent predictors of new PPM Table 3.

Table 3. Univariate and multivariate analysis to identify predictors of conduction disturbances requiring PPM at 30 days.

Predictors	Univariate Analysis		Adjusted Regression Analysis	
	Odds Ratio (95% CI)	***p*-Value**	**Odds Ratio (95% CI)**	***p*-Value**
Preprocedural aspects				
EuroSCORE II	0.62 (0.40–0.95)	0.028		
RBBB	10.21 (3.01–239.8)	<0.001	26.343 (3.924–176.837)	0.001
Membranous septum length	0.34 (0.19–0.58)	<0.001	0.276 (0.132–0.576)	0.001
MAC ≥ moderate	3.12 (1.03–9.42)	0.043		
Procedural aspects				
Implantation depth at NCC	1.62 (0.16–2.25)	0.004	1.576 (1.020–2.435)	0.04
MS length minus implant depth	0.56 (0.41–0.76)	<0.001		

Abbreviations: EuroSCORE II = European System for Cardiac Operative Risk Evaluation II; MAC = mitral annular calcification; MS = membranous septum; NCC = non coronary cusp; RBBB = right bundle branch block.

An MS length of <2.85 mm (AUC = 0.85, (95%CI: (0.77, 0.93) and ID of >3.99 mm (AUC = 0.79, (95%CI: (0.68, 0.90)) were found to be the optimal cut-offs by ROC analysis for predicting new PPM requirements at 30 days, as shown in Figures 3 and 4.

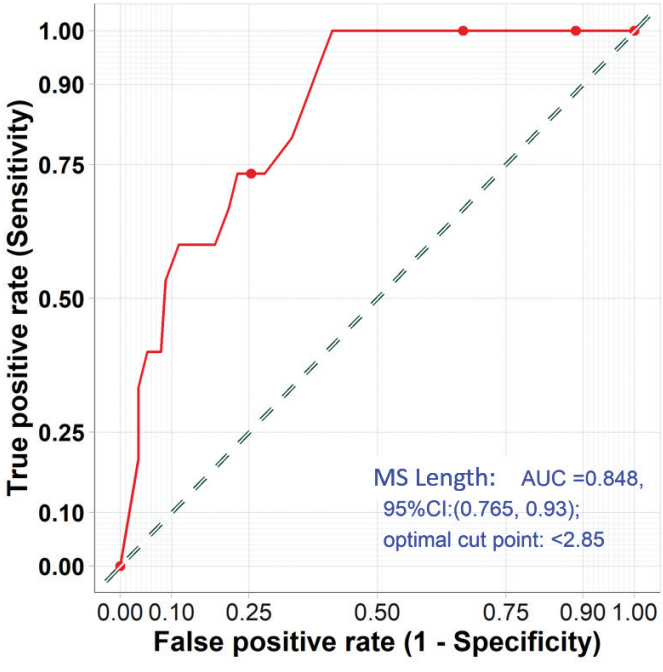

Figure 3. ROC results of the predictability power of membranous septum length for prediction of PPM.

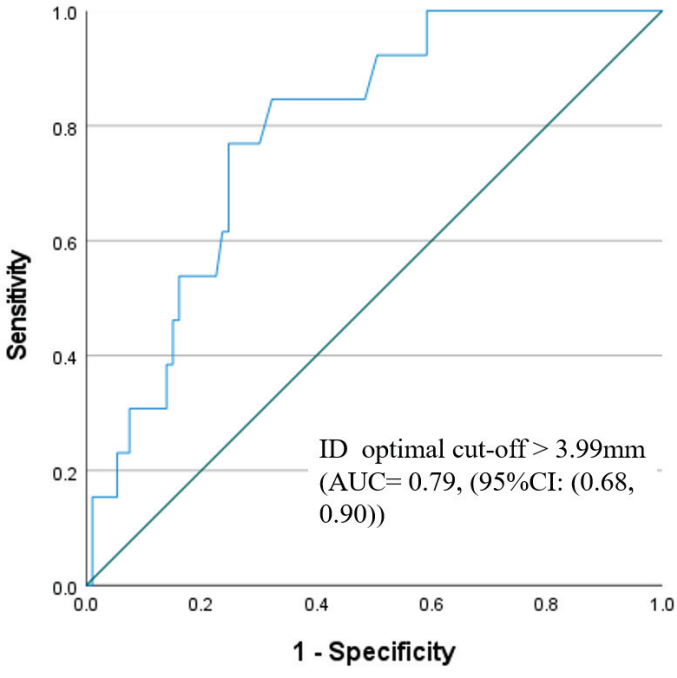

Figure 4. ROC results of the predictability power of implantation depth for prediction of PPM.

3.5. PR Interval and QRS Duration Changes from Baseline to Post-Procedure and Discharge

PR and QRS duration were calculated at three timepoints: immediately pre-TAVI, immediately post-TAVI and at discharge. The 15 patients who required new PPM post-TAVI were excluded from this analysis. The PR interval was prolonged post-TAVI [208 (178, 240) vs. 182 (164, 209) msec, $p = 0.002$], which was recovered at discharge [188 (171, 219) msec vs. 182 (164, 209) msec, $p = 0.064$]. On the other hand, the QRS duration was prolonged post-TAVI [127 (107, 150) vs. 101 (89, 119) msec, $p < 0.001$], which continued at discharge [125 (100, 150) msec, $p < 0.001$], as shown in Table 4 and Figure 5.

Table 4. Change in PR interval and QRS duration measured pre-TAVI, post-TAVI and pre-discharge.

	Time	Median [IQR]	*p*-Value
PR interval (msec)	Pre TAVI *	182 [164, 209]	-
	Post TAVI	208 [178, 240]	0.002
	At discharge	188 [171, 219]	0.064
QRS dutaion (msec)	Pre TAVI *	101 [89, 119]	-
	Post TAVI	127 [107, 150]	<0.001
	At discharge	125 [100, 149]	<0.001

* Reference category. Abbreviations: IQR = interquartile range; msec = millisecond; TAVI = transcatheter aortic valve implantation.

3.6. Procedural and Short-Term Outcomes

Procedural and in-hospital death occurred in 0% and 2% of patients, respectively. In-hospital deaths were attributed to stroke, right ventricular failure, and intestinal ischemia.

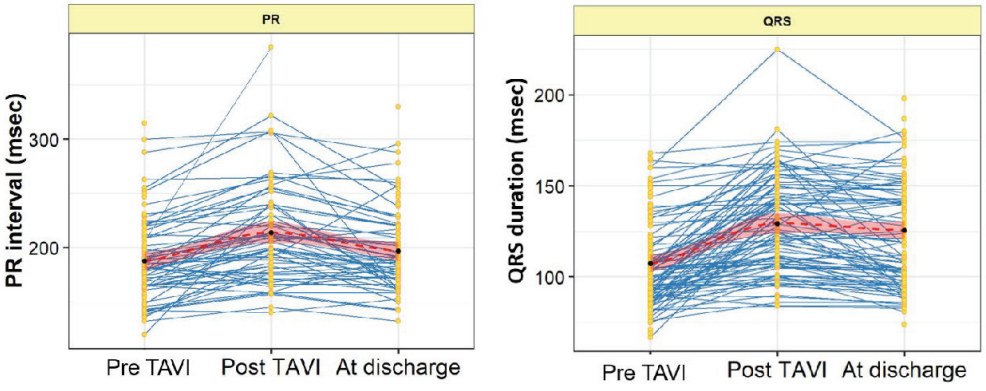

Figure 5. PR interval and QRS duration measurements pre-TAVI, post-TAVI and pre-discharge. The blue line represents the changes observed in each patient, while the red line depicts the average changes observed in all patients. Abbreviations: msec = millisecond; TAVI = transcatheter aortic valve implantation.

4. Discussion

The present study explored the predictors of new PPM in contemporary TAVI patients receiving Evolut platforms (R/PRO/PRO+). The main findings are as follows:

(1) At 30 days, the rate of new PPM implantation was 11.6%.
(2) On multivariate analysis, pre-existing RBBB, MS length, and ID were found to be the strongest predictors of new PPM.
(3) The optimal membranous septum length cut-off to predict new PPM was <2.85 mm AUC = 0.85, (95% CI: 0.77–0.93) while the optimal implantation depth cut-off was >3.99 mm and AUC = 0.79 (95% CI: 0.68–0.90).
(4) Detailed ECG analysis showed significant prolongation of the PR interval and QRS duration post-TAVI. The PR interval prolongation recovered pre-discharge, while QRS duration persisted until discharge compared to the baseline measurements.

Conduction abnormalities remain a significant hurdle to successful TAVI implantation. The close relation between His bundle and the left bundle branch to the aortic annulus explains this phenomenon. The conduction system injury is likely due to inflammation, oedema, or ischemia, which occur during TAVI implantation [5]. The His bundle course may be one of three anatomical variations: 50% penetrate the right side of the ventricular septum, 30% penetrate the left side and, infrequently, it courses under the membranous septum just below the endocardium (20%) [18]. These anatomical variations may explain the complexity of conduction disturbance predictions.

The rate of new PPM in our study was 11.6%, which is consistent with new PPM rates in the studies on newer-generation Evolut platforms that have been published to date [11,13], but less than the rate of new PPM in the Evolut Low Risk Trial [1]. These data are interesting, as our cohort would be considered low-risk, with a median EuroSCORE II of 3.2. This difference may be explained by the use of older-generation devices in the Evolut Low Risk trial (CoreValve and Evolut R), while Evolut R accounted for ~30% of the valves included in this study, with the majority of implants being Evolut Pro/Pro+. Our study, therefore, adds weight to the observation of a steady decline in new PPM requirements with successive iterations of the Medtronic Evolut family of devices. The adoption of COT and high ID in our cohort may be contributed to the lower PPM rate.

Pre-existing RBBB has been recognized as the most consistent predictor of new PPM implantation and, again, our study affirms this finding. The other predictors, e.g., MAC severity, MS length and implantation depth, and its relation to the MS length, were frequently identified in other studies [4,9]. The INTERSECT registry analyzed the effect of MS

length on pacemaker requirements post-TAVI among 1811 patients, utilizing various TAVI devices. The study revealed that MS length was a significant predictor of PPM for all TAVI platforms, except for the ACURATE neo [19].

All these predictors are non-modifiable, except the implantation depth. Jilaihawi and colleagues [13] proposed that the high PPM achieved with the Evolut platform can be alleviated when aiming for a pre-release ID that is less than the MS length. The new PPM rate at 30 days was significantly lower in their prospective cohort using the suggested approach (3% vs. 9.7%, $p = 0.035$). Indeed, in our study, an ID greater than the MS length was also found to occur more frequently in those requiring a new PPM. The same approach, using a high deployment technique, was applied by Sammour et al. [20] for the implantation of balloon-expandable SAPIEN 3 valve, resulting in a significant reduction in the 30-days PPM post-TAVI (5.5% vs. 13.1%, $p < 0.001$). In a recently published meta-analysis on the use of MS length as a predictor of PPM after TAVI [21] and its interaction with the ID, including 18 studies, it was found that a short MS length and low difference between the MS length and the ID were associated with a higher risk of PPM post-TAVI.

Changes in the implantation technique are already underway with the Evolut family of devices. The wide variability in new the PPM requirements across previous studies with these platforms suggests the need for a standardization of implantation techniques. The incidence of new PPM post-TAVI in the Evolut Low Risk Trial, for example, ranged from 1.6% to 26.2% at the four highest implanting sites in the study [22]. The ongoing post-market Optimize PRO study (NCT04091048) aims to standardize implantation techniques using the cusp overlap view, paying particular attention to the implantation depth (targeting 3–5 mm). An interim analysis of North American sites found that the rate of new PPM implantations at 30 days was 9.8%, which significantly decreased to 5.8% when using the cusp overlap technique(COT) [23].

Use of the COT was numerically higher in patients who did not require a new PPM but statistically non-significant (67% vs. 47%, $p = 0.16$), which could be due to the relatively small sample size in our cohort.

On the other hand, the continuous improvement in the devices and their delivery systems led to a significant decrease in major periprocedural complications, including new PPM [7,10]. Initially, the original Medtronic CoreValve platform was approved for clinical use in Europe in 2007, followed by Evolut R in 2014, Evolut PRO and Evolut PRO+, and finally Evolut FX.

Evolut FX received FDA approval in August 2021 but is not yet approved in Europe. The Evolut FX has a more flexible delivery system to assist in the steering of the valve through complex anatomies and is equipped with three radiopaque markers to enhance visualization and improve position accuracy and commissural alignment [24]. Furthermore, the delivery system has an optimized stability layer for more predictable deployment. However, the initial results of first in human (FIH) [25] showed no statically significant difference between the Evolut FX ($n = 43$) and Evolut PRO+ ($n = 378$) regarding the rate of new PPM or new LBBB (7% vs. 11.2%, $p = 0.78$ and 16.3% vs. 10.6%, $p = 0.20$, respectively). Of note, the Evolut FX cohort had a significantly higher implantation (ID at NCC was 2.5 ± 2.3 vs. 3.4 ± 2.3, $p = 0.016$ and at LCC was 2.5 ± 2.3 vs. 3.4 ± 2.3, $p = 0.016$, respectively) with a higher rate of commissural alignment (93% vs. 80.2%; $p = 0.039$, respectively). Evidently, this study was a retrospective reporting the initial experience in a few numbers of patients treated with Evolut FX, which needs to be confirmed in prospective multicentre randomized studies.

There have been limited studies assessing the impact of TAVI on the cardiac electrical properties of patients who do not require a PPM after the procedure. In our study, both PR interval and QRS duration were significantly prolonged post-procedure in comparison to the baseline. The PR prolongation recovered while the QRS widening persisted at discharge. This is in contrast to other studies, which showed that the PR prolongation persisted at discharge [26,27]. After a six-month follow-up of 182 patients who underwent TAVI, it was observed that, while the QRS widening continued, the PR prolongation did not persist [26].

Predicting the need for new PPM following TAVI is possible with the presence of pre-existing RBBB, a short membranous septum and the presence of MAC on MSCT, which was significant in our study. Systematic measurement of the MS length during pre-procedure planning, aiming for a patient-specific implant depth, may be an important evolution in the implantation technique for these devices. These data should guide the procedural planning, and the discussion of the risk of new PPM should be integrated into the informed consent process with patients and during the institutional heart team discussion. Furthermore, it should be integrated into procedural planning, including device selection, implantation height, pre- and post-balloon dilation, choice of pacing strategy during the procedure, and the duration of post-procedure telemetry monitoring [9,28]. Finally, as a high THV implantation can potentially impede future access to the coronary arteries or render TAV-in-TAV procedures more challenging in a proportion of patients [11], the balance between avoiding a new PPM and facilitating future procedures should be carefully weighed on a case-by-case basis.

There are several important limitations. Our study is a single-centre retrospective study with a relatively small sample size, bearing the limitations inherent to this design. The average age of our patients was 81.3 years, and only 36% were female. It is important to consider this context when interpreting the study results. The measurement of ID from the final aortography may be affected by the angle of acquisition or the amount of injected contrast. Similarly, the presence of localized calcification or a narrow sinus makes the identification of the annular plane difficult. Post-TAVI MSCT can be the best option to obtain a precise ID measurement, which was not carried out in our study. Finally, we did not investigate the PPM dependence or the recovery of conduction after PPM implantation in our study.

5. Conclusions

In this single-centre retrospective study, the rate of new PPM implantation post-TAVI with the Evolut platform was 11.6% at 30 days. Membranous septum length and implantation depth were independent predictors of new PPM. A customized implantation depth based on the membranous septum length could mitigate the new PPM rate.

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Effects of Atrial Fibrillation Radiofrequency Ablation in Patients Aged > 75 Years Undergoing Mitral Valve Surgery

Carlo Rostagno [1,*], Camilla Tozzetti [1], Enrico Carone [2] and Pierluigi Stefàno [2]

[1] Dipartimento Medicina Sperimentale e Clinica, Università Firenze, 50134 Firenze, Italy
[2] Cardiochirurgia AOU Careggi Firenze, 50134 Firenze, France
* Correspondence: carlo.rostagno@unifi.it

Abstract: Background: Few data exist about the efficacy of radiofrequency (RF) maze procedures in elderly patients with atrial fibrillation (AF) undergoing surgery for mitral valve disease. The aim of the present investigation was to evaluate the effects of AF ablation associated with mitral valve surgery on the recovery and long-term maintenance of sinus rhythm in elderly patients aged > 75 years. Moreover, we evaluated the effects on survival. Methods and results: This study included 96 consecutive patients with AF (42 men and 56 women) aged > 75 years (mean age 78 ± 3) who underwent RF ablation associated with mitral valve surgery (group I). This group was compared to 209 younger patients (mean age 65 ± 8 years) treated in the same period (group II). Baseline clinical and echocardiographic characteristics were similar in the two groups. Four patients died during hospitalization, one aged > 75 years. In surviving patients at the end of the follow-up period, sinus rhythm was present respectively in 64% of the elderly and 74% of younger patients ($p = 0.778$). The rate of persistence of sinus rhythm without AF recurrences (38% vs. 41%, $p = 0.705$) was similar in the two groups. After surgery, sinus rhythm was frequently never regained in aged patients (27% vs. 20%, $p = 0.231$). Elderly patients more frequently needed permanent pacing and had more hospitalizations and a higher number of non-AF atrial tachyarrhythmias. At eight-year follow-up, survival was lower in older patients (48% aged > 75 vs. 79% aged < 75 years). Conclusion: Elderly patients had a similar long-term rate of stable sinus rhythm maintenance in comparison to younger patients after AF radiofrequency ablation associated with mitral valve surgery. However, they needed more frequent permanent pacing and had higher rates of hospitalizations and post-procedural atrial tachyarrhythmias. The effects of survival are difficult to evaluate due to the different life expectancies of the two groups.

Keywords: mitral valve surgery; atrial fibrillation; radiofrequency ablation; heart failure; rheumatic valve disease; survival

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1. Introduction

In the last decade, due to population aging, the need for heart surgery in elderly patients (>75 years) has increased significantly [1]. A higher prevalence of AF in elderly people is a long-term recognized phenomenon [2]. After mitral valve surgery without ablation, spontaneous rhythm restoration occurs in no more than 20% of patients. The persistence of atrial fibrillation (AF) is associated with decreased functional capacity, an increased risk of embolization [3–5], and higher mortality [6,7]. Radio-frequency ablation has been consistently demonstrated to restore sinus rhythm in patients undergoing mitral valve surgery [8–10]. The long-term success rate is influenced by several variables, for example, completeness of line ablation, left atrium dimensions, concomitant surgery other than mitral valve repair/replacement, and finally, rheumatic etiology. Moreover, different results were reported in patients treated with mono- or bipolar techniques [11]. Little information is available about the effects of age since almost all studies included patients aged < 70 years. Catheter ablation of atrial fibrillation has been safely and successfully

performed in elderly patients with and without underlying heart valve disease who do not need surgical treatment with results similar to younger patients.

The aim of the present investigation was to prospectively assess the effects of monopolar-bipolar radiofrequency ablation of AF performed during mitral valve surgery in patients aged > 75 years compared with a younger control group. At an average 8-year follow-up, the rate of persistence of sinus rhythm and the frequency of clinically documented recurrences of AF were compared between the groups. We also compared the need for further hospitalization due to cardiac events and overall survival. Finally, in surviving patients. we examined the relationship between the persistence of sinus rhythm and functional capacity.

2. Materials and Methods

2.1. Patient Population

Between January 2010 and December 2015, monopolar or bipolar radiofrequency ablation associated with mitral valve surgery was performed at the Heart Surgery Department of the Azienda Ospedaliera Universitaria di Careggi (AOU) in 301 patients with AF. Informed consent was obtained before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of AOU Careggi.

Functional capacity was expressed as NHYA functional class. All patients underwent transthoracic echocardiography (Sequoia C256 Accuson Siemens, Mountain View, CA, USA). In each patient, the following dimensions were measured: left atrium AP diameter (mm), 2D left and right atrium area (cm^2), and left ventricular ejection fraction (LVEF). Since most patients were in AF, we considered the average value of five measurements. End-diastolic and end-systolic images were synchronized on ECG. Pulmonary systolic pressure (PAP) was calculated by adding the RV/RA pressure gradient to the estimated right atrial pressure assessed by inferior vena cava diameter and response to respiratory acts.

The follow-up of this prospective study was conducted as outpatients with clinical, electrocardiographic, and echocardiographic examinations at one and six months and thereafter yearly. The overall duration of follow-up was 8 years.

2.2. Radiofrequency Ablation Procedure

Medtronic Cardioablate surgical ablation systems (Medtronic, Minneapolis, MN, USA) were used for monopolar and bipolar treatment. Access to the inside of the left atrium was gained through a standard atriotomy. After left atrial appendage (LAA) excision, ablation lines were performed. A detailed description of left-sided ablation lines has been previously reported [12]. The amount of cardiopulmonary bypass time required for ablation was, on average, 15 ± 7 min.

2.3. Postoperative Management

Standard antiarrhythmic prophylaxis consisted of i.v. and thereafter orally administered amiodarone according to a previously reported protocol [12]. Patients with persistent AF despite optimal medical therapy before discharge underwent at least one attempt of external cardioversion with biphasic DC shock. Oral anticoagulation was given to maintain the international normalized ratio between 2.5 and 3.5 for the first 6 months in all patients and for life in patients who received mechanical valves or who had AF persistence, or both.

2.4. Follow-Up

Follow-up visits were performed at 3, 6, and 12 months after surgery and annually thereafter. Between visits, their referring physician followed patients on a regular basis, and routine ECGs were obtained at each clinic visit regardless of symptoms. Between visits, all patients were encouraged to seek 12-lead ECG documentation for any symptom suggestive of AF/atrial flutter recurrence, and a physician routinely performed trans-telephonic monitoring of any symptoms and complications.

The follow-up evaluation consisted of a detailed history, physical examination, and 24-h Holter monitoring. Success and AF recurrence were defined following the HRS/EHRA/ECAS expert consensus document [13].

2.5. Statistical Analysis

Continuous variables were presented as means ± SD, while categorical variables were reported as percentages. Continuous variables were compared with Student's 2-tailed unpaired samples *t*-test. Categorical variables were compared using the chi-squared test or Fisher's exact test if appropriate. Kaplan–Meier curves were used for the survival analysis. Differences between groups were compared using the log-rank test.

A probability value <0.05 was considered significant. Statistical analyses were performed with SPSS 22.0 software (SPSS, Inc., Chicago, IL, USA).

3. Results

The study included 95 patients (54 men and 42 women, mean age 78 ± 3 years). This group was compared to 206 younger patients (111 males and 95 females, mean age 65 ± 8 years). The characteristics of the two groups are reported in Table 1. The duration of atrial fibrillation was significantly longer in younger patients (54 vs. 26 months). Pulmonary pressure was slightly higher in elderly patients, while at the time of surgery, the degree of functional impairment (most patients in the III-IV NHYA class) did not differ between the two groups.

Table 1. Clinical characteristics of patients.

	>75 Years ***n* = 96**	**<75 Years** ***n* = 209**	
Age (years)	78 ± 3	65 ± 8	<0.001
Gender (male/female)	54/42 (56%)	111/95 (53%)	0.774
Persistent AF/paroxysmal AF	81/15 (83%)	183/26 (87%)	0.475
AF duration before surgery (months)	26 ± 28	54 ± 34	<0.001
Left ventricular EF (%)	52 ± 7	53 ± 9	0.334
Left atrium diameter (mm)	47 ± 7	46.5 ± 7	0.383
Left atrium area (cm^2)	31.0 ± 7	32.7 ± 10.6	0.156
Right atrium area (cm^2)	21.4 ± 5	21.2 ± 7	0.854
Systolic pulmonary pressure (mmHg)	47 ± 15	43 ± 14	0.035
NHYA class	3.1 ± 0.7	3 ± 0.55	0.177

AF = atrial fibrillation, EF = ejection fraction, NYHA = New York Heat Association.

The etiology of mitral valve disease is reported in Table 2. No significant differences were found between younger and elderly patients. Rheumatic disease was still the prevalent indication for mitral valve surgery in both groups, though valve prolapse accounted for about a quarter of patients in elderly and younger patients.

Table 2. Etiology of mitral valve disease.

	>75 Years ***n* = 96**	**<75 Years** ***n* = 209**	***p***
Mitral valve prolapse	22 (23%)	51 (24%)	0.885
Rheumatic mitral valve disease	25 (26%)	64 (31%)	0.494
Mitro-aortic rheumatic valve disease	18 (19%)	55 (26%)	0.193
Ischemic mitral regurgitation	17 (17.2%)	21 (10%)	0.072
Mitral regurgitation associated with DCM	13 (14%)	14 (7%)	0.063
Other (including tricuspid valve repair)	1 (0.8%)	4 (2%)	0.823

DCM—dilated cardiomyopathy.

Surgical techniques are reported in Table 3. Isolated procedures on the mitral valve were performed in 43% of elderly patients in comparison to 53% of the control group. Mitral valve replacement was more frequently performed in younger patients, while tricuspid valve repair for severe tricuspid regurgitation was more frequently performed in elderly patients (most, >85%, were performed according to the Kay technique). Mitral regurgitation secondary to coronary heart disease was present in 17.3% of the elderly vs. 10% of the control group. Radiofrequency ablation was performed using a unipolar probe in 56% of cases, and bipolar ablation was performed in the remaining 44% of cases. The proportion of patients undergoing monopolar vs. bipolar ablation did not differ between elderly and younger patients. Furthermore, the clinical characteristics of patients undergoing the two techniques were similar between the subgroups.

Table 3. Intervention performed.

Intervention	>75 Years n = 96	<75 Years n = 209	p
Mechanical mitral valve replacement	8 (9%)	39 (19%)	0.025
Mitral valve repair	32 (34%)	71 (34%)	0.910
Mitral and aortic valve replacement	15 (16%)	42 (20%)	0.422
Mitral valve repair and CABG	20 (21%)	31 (15%)	0.196
Mitral valve replacement and CABG	4 (2%)	9 (5%)	0.445
Other (including tricuspid valve repair)	17 (18%)	14 (7%)	0.007

CABG = Coronary artery by-pass grafting.

3.1. Rhythm Analysis

A total of 60/92 subjects (65%) aged > 75 years were in sinus rhythm at hospital discharge in comparison to 143/209 (68%) of younger patients (p = 0.360). In surviving patients at the end of the follow-up period, sinus rhythm was present in 64% of elderly and 74% of younger patients (p = 0.778). Sinus rhythm never recovered after ablation and electrical CV attempts in 27 (28%) individuals aged > 75 years and in 45 (21%) younger patients (p = 0.231). The recurrence rate was 32% and 35% in the two groups (p = 0.705) (Table 4). In both groups, the persistence of stable sinus rhythm was less frequent in patients with rheumatic valve disease. Additionally, these patients had a higher rate of atrial fibrillation recurrence. We did not find any significant difference in long-term results in patients treated with monopolar or bipolar ablation.

Table 4. Long-term results of AF ablation.

	Stable Sinus Rhythm	AF Recurrences	Never Recovered Sinus Rhythm	p
Aged < 75 years	86	75	45	0.822
Aged > 75 years	37	31	27	

In elderly patients, atrial tachyarrhythmias different from atrial fibrillation were more frequent (11.5% vs. 4%, p = 0.025) than in younger patients. Permanent pacing was also more frequently needed in the older group (22 vs. 11%, p = 0.014) (Table 5).

Ischemic stroke occurred in six patients (2%) during the follow-up period, with 3 in each group. In patients with permanent AF, at the moment of the stroke, 3 had INR values below the therapeutic range. One of the two patients in SR had severe carotid stenosis. A higher rate of hospitalization due to cardiac causes during the follow-up period was found in the elderly group (41 vs. 30%, p = 0.004).

Table 5. Complications.

Complications	>75 Years	<75 Years	*p*
Definitive pacemaker	21 (22%)	23 (11%)	0.014
Hospitalizations	45 (41%)	62 (30%)	0.004
Other atrial arrhythmias	11 (11.5%)	9 (4%)	0.025
Stroke	3 (3.1%)	3 (1.4%)	0.38

3.2. Functional Capacity

The baseline mean NYHA class was 3 in both groups before surgery and ablation. A significant improvement in functional capacity was found in patients in sinus rhythm at the end of follow-up (both patients with stable sinus rhythm and with AF recurrences) but not in patients who never recovered sinus rhythm (mean NYHA class was, respectively, 1.3 ± 0.4 vs. 2.3 ± 0.6, $p < 0.001$)

3.3. Mortality

At eight years of follow-up, overall survival was 78% (Figure 1). Eighty-eight patients died: in 62%, death was due to cardiac causes, and in the other 38%, the cause of death was not cardiac or unknown. Mortality was close to 50% in patients aged > 75 years in comparison to 20% in younger patients. Survival curves, however, began to diverge only after the first 1500 days of follow-up, a phenomenon related to the decreased life expectancy of elderly patients. Mortality in patients with rheumatic disease was higher than in those suffering from mitral valve prolapse (22% vs. 8%, $p = 0.01$). Tricuspid valve repair, more frequently performed in aged patients with pulmonary hypertension, was associated with significantly higher mortality. Preoperative NHYA class was not related to survival both in elderly and younger patients, while failure to restore sinus rhythm with RF ablation was related to a worse prognosis independently of age. Mortality was 44% in patients in AF who never recovered SR in comparison to 16% of those in stable sinus rhythm after discharge. There was no difference in survival rate between patients with stable sinus rhythm during follow-up and patients with AF recurrences. Finally, no differences in mortality were observed between patients treated with monopolar or bipolar ablation.

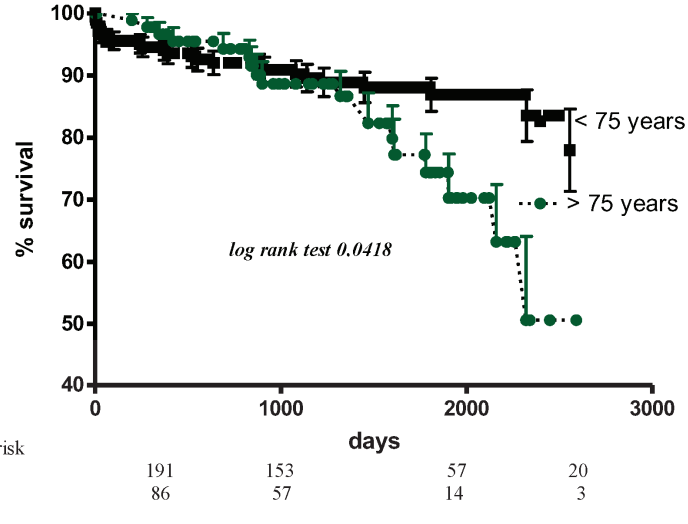

Figure 1. Kaplan–Meier survival curves according to age.

4. Discussion

The increasing number of aged patients who need heart surgery and suffer from atrial fibrillation raises the question of the cost-effectiveness of ablative procedures in elderly patients [13]. A recent study demonstrated that radiofrequency ablation in patients with heart failure and atrial fibrillation significantly decreased the combined endpoint of death and hospitalization for worsening heart failure [14]. Death due to cardiovascular causes was two-fold higher in patients treated with medical therapy in comparison to patients who underwent ablation. Non-treated atrial fibrillation in patients undergoing valve surgery is associated with a higher risk of stroke and mortality, and age has been demonstrated as a relevant independent factor for poorer outcomes [3,13]

Several studies, including matched-controlled and randomized trials [15], have consistently demonstrated that radiofrequency ablation associated with mitral valve surgery, but also with other surgical procedures, maintains sinus rhythm at short- and long-term follow-up [16,17], decreases the risk of stroke, and, with a lower degree of evidence, improves long-term survival. The rate of clinical success, however, is significantly influenced by the population selected [18,19]. Female gender, duration of atrial fibrillation above 24 months, increased left atrial dimensions (LA M-mode diameter > 54 mm, left atrial area > 24 cm^2), rheumatic valve disease, and NYHA class were associated with a higher rate of ablation failure [20].

Analysis of the wide range of literature published in the last 15 years, however, shows that in most published studies, the mean age of included patients was between 55 and 65 years; therefore, results are not applicable to the elderly population.

Extensive evidence exists at present indicating that catheter ablation of atrial fibrillation can be safely and successfully performed in the elderly with and without underlying heart valve disease who do not need surgical treatment. Wang et al. [21], in a propensity score study, matched 347 pairs of patients aged > 75 years undergoing or not undergoing ablation. Ablation was associated with a lower risk of a composite outcome of all-cause death, non-fatal stroke, and peripheral embolism (hazard ratio (HR) = 0.40; 95% confidence interval (CI): 0.19–0.85), all-cause death (HR = 0.13 95% CI: 0.04–0.43), and major bleeding (HR = 0.23; 95% CI: 0.12–0.67). Nademanee et al. [22] evaluated 587 elderly patients (age $\geq$ 75 years) with AF. Three hundred and twenty-four were eligible for ablation. The 261 (group 1) who underwent ablation were compared with the other 63 patients (group 2) who declined or were not suitable for ablation. Normal sinus rhythm, stroke, death, and major bleeding were the main endpoints. At a mean follow-up of 3 $\pm$ 2.5 years 216 (83%) of group 1 patients remained in sinus rhythm in comparison to 14 of group 2 patients (22%, $p < 0.001$). At five years, survival was 87% in patients in sinus rhythm, 52% in patients with AF, and finally, 42% in patients who did not undergo ablation. Overall, the efficacy rates of catheter ablation in restoring sinus rhythm were reported to be between 75 and 85% [23–25]. High rates of atrial fibrillation control in the elderly were obtained despite the higher prevalence of structural heart disease and the higher prevalence of persistent atrial fibrillation. The efficacy endpoints, however, were not uniform, and in many studies, effective rhythm control required the continued use of antiarrhythmics post-ablation [23–25]. Nevertheless, results of catheter ablation in elderly patients suggest that age is not a contraindication to treatment.

Less is known about ablation associated with heart and, in particular, mitral valve surgery. Ablation is performed in less than 60% of patients with AF undergoing mitral valve surgery, and multivariate regression has demonstrated that age and comorbidities are strong predictors of a lower probability of performing concomitant AF ablation [26]. In their study, Petersen et al. [27] reported that freedom from atrial fibrillation at 12 months after surgery associated with AF ablation was between 62% and 72%. This was independent of age except for elderly patients undergoing concomitant coronary artery bypass grafting surgery. Double-valve procedures (odds ratio, 3.48; $p = 0.020$), preoperative persistent atrial fibrillation (odds ratio, 2.43; $p = 0.001$), and coronary artery bypass grafting surgery in elderly patients (odds ratio, 2.03; $p = 0.009$) were risk factors for the recurrence of atrial fibrillation. Lin et al. [28] evaluated the effects of bipolar radiofrequency ablation in patients

aged > 65 years undergoing mitral valve replacement according to frailty status. Even if freedom from AF after 1 year was not different in the frail group compared to the non-frail group (75.1% vs. 73.5%), the frail group had a higher adjusted risk for all-cause mortality and all-cause hospitalization. Rates of cardiovascular death, stroke or non-CNS embolism, and cardiovascular hospitalization were similar between the two groups.

The study by McGregor et al. [29] compared elderly patients (mean age 78.5 ± 2.8 years) undergoing MV repair or replacement with a younger group. In elderly patients, MV replacement was more frequent than repair; additionally, elderly patients more frequently underwent other surgical procedures concomitantly. Baseline clinical conditions were more compromised in the elderly group (lower BMI, higher rates of hypertension, previous myocardial infarction, and heart failure). Major complications after surgery and 30-day mortality were more frequent in the elderly (23% vs. 14%, $p = -0.017$ and 6% vs. 2%, $p = 0.026$, respectively). Freedom from atrial fibrillation and antiarrhythmic drugs (AADs) was lower in elderly patients at 4 years (65% vs. 79%, $p = 0.043$).

Results from the present prospective study suggest that at the end of an eight-year follow-up period, the number of patients in stable sinus rhythm was non-significantly different between the two groups. Furthermore, the number of patients with AF recurrences and those who never regained sinus rhythm after surgery were similar in patients aged > 75 years and in the younger group. These data suggest that advanced age is not to be considered a contraindication to RF ablation during surgery for mitral valve disease; moreover, the results on rhythm control during follow-up are not different from those observed in younger patients. All patients had left atrial auriculectomy, and this may explain the small number of embolic complications (six, three for each group) found in the present study. Among patients in AF, 3/4 had INR values below the therapeutic range, while a critical stenosis of the internal carotid artery was found in one of the two patients in sinus rhythm.

Elderly patients had a two-fold higher need for permanent pacing than younger patients. Additionally, they needed more frequent hospitalizations and had a higher number of atrial tachyarrhythmias other than atrial fibrillation. The need for permanent pacing in patients aged > 75 years is consistent with previous data both after surgical [30,31] and catheter ablation of atrial fibrillation. In the study by De Rose et al., 14.4% of patients received a PPM within the first year after ablation associated with mitral valve surgery. A pacemaker was implanted in 7.7% of patients randomized to mitral valve surgery alone, 16.1% of patients who received mitral valve surgery + pulmonary vein isolation, and 25% of patients who received mitral valve surgery + a bi-atrial maze. Ablation, multivalve surgery, and New York Heart Association functional (NYHA) functional class III/IV were independent risk factors for PPM implantation. The need for PPM was associated with a higher risk of 1-year mortality (HR: 3.21; 95% CI: 1.01 to 10.17; $p = 0.05$) after adjustment for randomization assignment, age, and NYHA functional class. Higher rates of hospitalization and atrial arrhythmias other than atrial fibrillation were more frequently reported in elderly patients. More advanced atrial myopathy in older individuals may lead to a more complex substrate, which may affect the probability of the onset of atrial arrhythmias other than atrial fibrillation.

More difficult to evaluate is whether AF ablation may improve survival in elderly patients. Several studies have suggested increased survival in elderly patients after AF ablation in comparison to valve surgery alone [31]. Additionally, the persistence of sinus rhythm after surgery was associated with an improvement in functional capacity and increased survival in comparison to patients who remained in permanent AF. In a previous investigation from our group, mortality at five years was 30% in patients with permanent AF; otherwise, survival was not significantly different (close to 90%) between patients with stable SR and those in whom SR was present at the last follow-up visit, despite clinical documentation of at least a recurrence of AF requiring cardioversion [32]. Results from the present study show that survival curves between the two groups do not diverge within the first 1500 days after surgery. Most previous papers dealing both with catheter and surgical

treatment had a limited follow-up duration (no more than 4 years), which was half of the length considered in this study; therefore, age-related increases in mortality may have been overlooked. In our opinion, the increase in late mortality in elderly patients may be mainly related to the physiological decrease in life expectancy rather than to the effects of surgery and AF ablation.

Limitations

The main limitation of this prospective study is that it was conducted in a single high-volume center, and patients did not undergo randomization before surgery; therefore, both elderly and younger patients lack a control group. Therefore, we did not exclude possible confounding variables that may influence the results of the ablation procedure and the determination of clinical outcomes. It is impossible to know how many of the patients would convert to sinus rhythm spontaneously, and, more relevantly, it is impossible to know the effects of confounding variables (e.g., the different degrees of hemodynamic impairment). For example, tricuspid repair is associated with a lower rate of sinus rhythm restoration and with increased long-term mortality. In patients with severe pulmonary hypertension and right ventricular dysfunction, indications for tricuspid valve repair should be accurately evaluated. The overall limited number of patients undergoing isolated mitral valve repair or replacement does not allow us to assess whether a technical approach to mitral valve surgery may affect the results of the ablation procedure. Finally, although we did not find any significant difference between the results obtained with monopolar and bipolar ablation, again, the absence of randomization does not allow us to draw definite conclusions about the different results of radio-frequency ablation techniques.

5. Conclusions

Results from the present investigation support the efficacy of RF ablation (mono and bipolar) in restoring SR in patients with AF undergoing mitral valve surgery. We did not find significant differences between patients aged > 75 years and younger patients in terms of maintenance of SR and the recurrence rate of atrial fibrillation. Embolic complications are negligible in both groups. Aged patients have a two-fold risk of need for definitive pacing and a higher rate of atrial tachyarrhythmias, as well as a higher rehospitalization rate. The length of follow-up does not allow us to draw conclusions on the effects of the procedure on survival since the divergence of survival curves after the first 1500 days of follow-up may be related only to the decreased life expectancy of elderly patients.

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Subtotal Nephrectomy Associated with a High-Phosphate Diet in Rats Mimics the Development of Calcified Aortic Valve Disease Associated with Chronic Renal Failure

Hind Messaoudi [1], Thomas Levesque [1,2], Nicolas Perzo [1], Elodie Berg [1,3], Guillaume Feugray [1,4], Anaïs Dumesnil [1], Valéry Brunel [4], Dominique Guerrot [1,5], Hélène Eltchaninoff [1,2], Vincent Richard [1,6], Saïd Kamel [7,8], Eric Durand [1,2], Youssef Bennis [1,7,9] and Jérémy Bellien [1,6,*]

1 INSERM EnVI UMR 1096, University of Rouen Normandie, F-76000 Rouen, France
2 Department of Cardiology, CHU Rouen, F-76000 Rouen, France
3 Department of Thoracic Surgery, CHU Rouen, F-76000 Rouen, France
4 Department of General Biochemistry, CHU Rouen, F-76000 Rouen, France
5 Department of Nephrology, CHU Rouen, F-76000 Rouen, France
6 Department of Pharmacology, CHU Rouen, F-76000 Rouen, France
7 UR UPJV 7517, Mécanismes Physiopathologiques et Conséquences des Calcifications Cardiovasculaires (MP3CV), Centre de Recherche Universitaire en Santé, Université de Picardie Jules Verne, F-80054 Amiens, France
8 Department of Biochemistry, Amiens-Picardie University Hospital, F-80054 Amiens, France
9 Department of Pharmacology, Amiens-Picardie University Hospital, F-80054 Amiens, France
* Correspondence: jeremy.bellien@chu-rouen.fr; Tel.: +33-(0)2-35-14-83-68

Abstract: Introduction. This study addressed the hypothesis that subtotal nephrectomy associated with a high-phosphorus diet (5/6Nx + P) in rats represents a suitable animal model to mimic the cardiovascular consequences of chronic kidney disease (CKD) including calcified aortic valve disease (CAVD). Indeed, the latter contributes to the high morbidity and mortality of CKD patients and sorely lacks preclinical models for pathophysiological and pharmacological studies. **Methods.** Renal and cardiovascular function and structure were compared between sham-operated and 5/6 Nx rats + P 10 to 12 weeks after surgery. **Results.** As expected, 11 weeks after surgery, 5/6Nx + P rats developed CKD as demonstrated by their increase in plasma creatinine and urea nitrogen and decrease in glomerular filtration rate, estimated by using fluorescein-isothiocyanate-labelled sinistrin, anemia, polyuria, and polydipsia compared to sham-operated animals on a normal-phosphorus diet. At the vascular level, 5/6Nx + P rats had an increase in the calcium content of the aorta; a decrease in mesenteric artery dilatation in response to a stepwise increase in flow, illustrating the vascular dysfunction; and an increase in blood pressure. Moreover, immunohistology showed a marked deposition of hydroxyapatite crystals in the aortic valve of 5/6Nx + P rats. Echocardiography demonstrated that this was associated with a decrease in aortic valve cusp separation and an increase in aortic valve mean pressure gradient and in peak aortic valve velocity. Left-ventricular diastolic and systolic dysfunction as well as fibrosis were also present in 5/6Nx + P rats. **Conclusion.** This study demonstrates that 5/6Nx + P recapitulates the cardiovascular consequences observed in humans with CKD. In particular, the initiation of CAVD was shown, highlighting the interest of this animal model to study the mechanisms involved in the development of aortic stenosis and test new therapeutic strategies at an early stage of the disease.

Keywords: animal model; chronic kidney disease; cardiovascular complications; calcified aortic valve disease

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1. Introduction

Despite major therapeutic advances, cardiovascular morbidity and mortality remain high in patients with chronic kidney disease (CKD) mainly due to the deleterious effects

of uremic toxins on the cardiovascular system [1,2]. Among their effects, the potentiation of the development of ectopic calcification in arteries but mostly in cardiac valves remain poorly studied due in particular to the lack of representative animal models [2–4]. Calcified aortic valve disease (CAVD) is a slow and progressive disorder that ranges from mild valve thickening without obstruction of blood flow, termed aortic sclerosis, to severe calcification with impaired leaflet motion that characterize aortic stenosis. CAVD is highly prevalent and aortic stenosis progresses more rapidly in CKD patients than in the general population, contributing to increase cardiac afterload that further aggravates cardiac remodeling and dysfunction and significantly reduces survival [3,4]. To date, the mechanisms involved in the development of CAVD are poorly understood, and no specific therapeutic strategies are available. This is notably due to the absence of adequate animal models that truly mimic human AS [5,6]. Recently, it was proposed that the induction of CKD using subtotal nephrectomy associated with a high-phosphorus diet (5/6Nx + P) can promote aortic valve calcification, but the associated modifications in cardiovascular structure and function have to be evaluated [7].

In this context, the aim of the present study was to perform a comprehensive cardiovascular phenotyping of 5/6Nx + P rats with particular emphasis on the development of CAVD.

2. Methods

2.1. Animals and Experimental Procedures

All procedures were performed in accordance with the standards and ethical rules (CENOMEXA #24107). Twenty-four 10-week-old male Sprague–Dawley rats were purchased from Janvier Labs (Le Genest-Saint-Isle, France) and were aged until 18 weeks old, weighing 500–600 g. Then, 16 rats were submitted to a two-step surgical procedure performed by a single trained experienced operator (H.M.) in order to ensure reproducibility. The first step of the surgical procedure consisted of ligation of the upper branch of the left kidney artery, followed by a cauterization of the lower pole of the left kidney, leading to 2/3 of a non-functioning left kidney. One week later, the right kidney was removed, inducing 5/6 Nx. Then, 5/6 Nx rats were fed ad libitum a normal-calcium, high-phosphate diet (pellets containing 1% total calcium, 1.8% total phosphorus, Safe, Augy, France) until animal sacrifice 11 weeks after surgery. A group of 8 sham-operated rats (surgical laparotomy) eating a standard normal-calcium, normal-phosphate diet (pellets containing 1% total calcium, 0.9% total phosphorus, Safe) served as controls.

2.2. Blood and Renal Evaluations

At sacrifice, plasma blood samples were collected to assay creatinine, urea nitrogen, sodium, potassium, calcium, and phosphorus on Cobas® analyzer (Roche, Mannheim Germany), as well as white and red blood cell counts, hemoglobin, and hematocrit levels. Uremic toxins including indoxyl sulfate, paracresyl sulfate, indole-acetic acid, trimethylamine oxide, and hippuric acid were assayed in the serum using a liquid chromatography–tandem mass spectrometry method as previously described [8]. Next, 24 h urine was collected 8 weeks after surgery using metabolic cages, allowing for the quantification of sodium, potassium, calcium, and phosphorus excretion, as well as albuminuria and creatininuria using the Catalyst Analyzer (IDEXX, Westbrook, ME, USA). Transcutaneous determination of glomerular filtration rate was performed 8 weeks after surgery by using fluorescein-isothiocyanate-labelled sinistrin as previously described [9].

2.3. Vascular Evaluations

Non-invasive measurements of systolic blood pressure were performed by tail cuff plethysmography (CODA, Kent Scientific Corporation, Torrington, CT, USA) 10 weeks after surgery. These measurements were performed in conscious and trained mice and consisted of two series of 10 cycles of measurements for each animal. At sacrifice, endothelium-dependent flow-mediated dilation was assessed on the second mesenteric-resistance artery

segment. Briefly, the mesentery was removed and placed in cold oxygenated Krebs buffer. A 2–3 mm segment of third mesenteric resistance artery segment was isolated and mounted on an arteriograph (DMT, Aarhus, Denmark). Vessels were pre-constricted using 10^{-5} M phenylephrine before assessing the dilatory response to stepwise increase in intraluminal flow (0, 5, 10, 25, 50, 100, and 150 μL/min). Endothelium-independent dilatation to sodium nitroprusside (10^{-5} M) was assessed in preconstricted vessels. In addition, the thoracic aorta was removed and cut in half. One segment was washed once with PBS and then decalcified with 0.6 N HCl overnight at 4 °C. The calcium content in the HCl supernatant was colorimetrically analyzed by the o-cresolphthalein complexone method [10]. Calcium content in aortic rings were corrected by aortic dry weight with aortas dried overnight at 37 °C. In addition, calcium deposition was evaluated on the second thoracic segment using 7 μm thick histological slices stained with Alizarin red [11].

2.4. Cardiac Evaluations

For transthoracic echocardiography, rats were anesthetized with isoflurane 2% in 21% oxygen/compressed air at 1 L/min) and placed on a heated plate to maintain body temperature at 37.5 °C, the chest shaved, and a Vivid 7 ultrasound echograph (GE Healthcare, Buc, France) equipped with a M12L linear probe operating at 14 MHz and fitted out with Echopac PC software (GE Medical Systems) was used [10].

2.4.1. Left-Ventricular Systolic Function

Briefly, a two-dimensional parasternal long-axis view of the left ventricle was obtained at the level of the papillary muscle, in order to record M-mode tracings. Left-ventricular (LV) end-diastolic (EDD) and systolic diameters (ESD) and end-diastolic anterior and posterior LV wall thicknesses were measured by the American Society of Echocardiology leading-edge method from at least 3 consecutive cardiac cycles. LV fractional shortening (FS) was calculated from the variation in LV diameters as FS (%) = ((LVEDD − LVESD)/LVEDD) × 100, and the LV ejection fraction (EF) was calculated by the Teicholz formula from LV diameters. Cardiac output (CO) was calculated by multiplying the stroke volume (the LV end-diastolic volume minus LV end-systolic volume) by the heart rate.

2.4.2. Left-Ventricular Diastolic Function

Doppler measurements were made at the tip of the mitral leaflets for diastolic filling profiles in the apical four-chamber view, allowing us to determine the E/e' ratio (mitral inflow E wave/e' tissue Doppler mitral annulus velocity) as an estimate of diastolic function.

2.4.3. Aortic Valve Assessment

In addition, the aortic valve peak flow velocity and aortic valve mean gradient were measured in the apical five-chamber view by continuous wave doppler in systole. The presence or absence of aortic regurgitation was visualized in this five-cavity view by color Doppler. Furthermore, aortic stenosis was estimated in two-dimensional parasternal long-axis view by measuring the aortic valve cusp separation in systole.

At sacrifice, the heart was harvested and weighed, and a section of the left ventricle was snap-frozen for subsequent determination of LV fibrosis, using 8 μm thick histological slices stained with Sirius Red as previously described [12]. In addition, the aortic valve was carefully dissected and incubated over 24 h at 4 °C in a solution of a 20 nM Osteosense 680Ex® (Perkin-Elmer, Waltham, MS, USA) in PBS. This fluorescent probe binds to hydroxyapatite with high affinity and thus allows for the detection of microcalcifications. Then, the valve was rinsed and snap-frozen for subsequent analysis using 8 μm thick histological slices with mounting medium containing DAPI. Pictures were acquired on an epifluorescence microscope (Axio Imager 1, Zeiss, Jena, Germany) equipped with an apotome using the Cyanine 5.5 filter for calcification detection and a DAPI filter for cell nuclei detection.

2.5. Statistics

All data are presented as mean ± SEM unless differentially indicated. Data normality was verified in sham-operated and 5/6Nx + P groups using the Shapiro–Wilk test. Comparisons between groups were performed using Student's t-test for normally distributed data and using the Wilcoxon rank-sum test for non-normally distributed data. The comparison between groups for endothelium-dependent relaxations to stepwise increase in flow was performed using repeated measures ANOVA.

3. Results

The mortality rate was 25% in the 5/6Nx + P group during the 11-week follow-up, while no death was observed in the sham-operated group (shown in Figure 1a). First of all, 5/6Nx + P rats developed renal failure, as shown by the increase in plasma urea and creatinine and the reduction of glomerular filtration rate compared to sham-operated rats (shown in Figure 1b–d). In addition, 5/6Nx + P rats had polyuria and polydipsia (shown in Figure 1e,f). Biochemical and hematological analyses are shown in Table 1. As expected from the experimental diet, phosphaturia was dramatically increased in 5/6Nx + P rats. Urinary calcium, sodium, and potassium levels were also all increased in 5/6Nx + P rats. However, the plasma levels of calcium and phosphorus as well as the calcium-phosphorus product remained similar between groups. In addition, the plasma sodium level was unchanged, but hyperkalemia was observed in 5/6Nx + P rats as well as hypoproteinemia. Furthermore, anemia developed in 5/6Nx + P rats as shown by the decrease in the number of blood erythrocytes and in hemoglobin and hematocrit levels without change in leukocyte number. As shown in Table 2, the concentrations of uremic toxins were also increased in the serum of 5/6Nx + P rats compared to sham-operated rats.

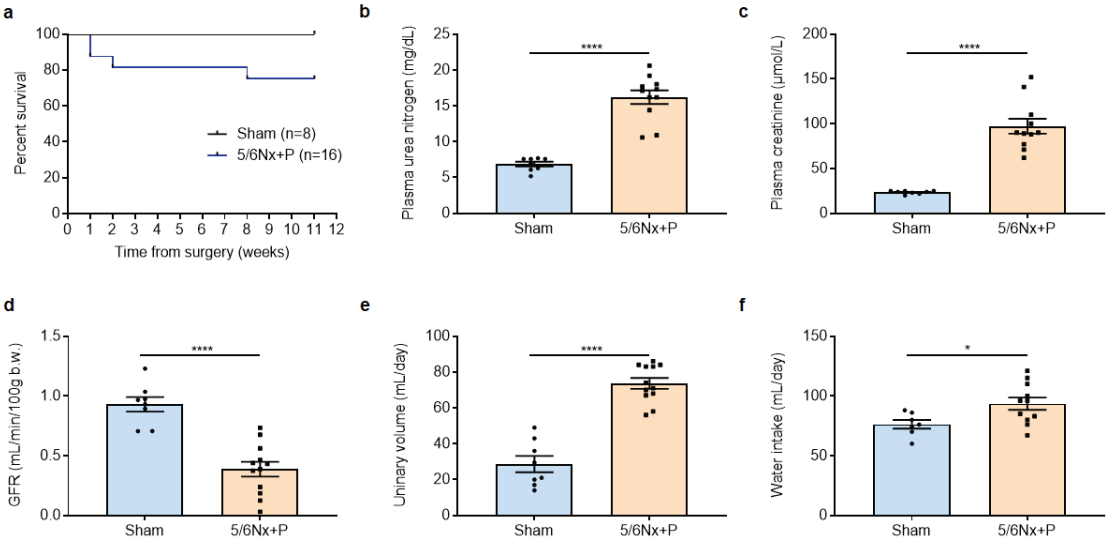

Figure 1. Eleven-week survival after surgery (**a**), plasma creatinemia (**b**), urea nitrogen (**c**), glomerular filtration rate (GFR, **d**), urinary volume (**e**), and water intake (**f**) measured 10 to 11 weeks after surgery in sham-operated and 5/6 nephrectomized rats receiving a high-phosphate diet (5/6Nx + P). * $p < 0.05$, **** $p < 0.0001$.

At the vascular level, 5/6Nx + P rats displayed aortic calcification, as shown by the increase in aortic calcium content and Alizarin red staining compared to sham-operated rats (Figure 2a,b). This was associated with the presence of systemic hypertension as well as

vascular dysfunction, as shown by the impairment of both mesenteric artery endothelium-dependent and -independent dilatation (shown in Figure 2c–e).

Table 1. Hematological and biochemical analyses, 9 to 11 weeks after surgery, in sham-operated rats and 5/6 nephrectomized rats receiving a high-phosphate diet (5/6Nx + P).

Parameters	Sham n = 8	5/6Nx + P n = 11 or 12	*p*-Value
Blood hematological parameters (at 11 weeks post-surgery)			
Leukocytes, $\times 10^9$/L	6.51 ± 0.46	5.78 ± 0.53	0.3655
Erythrocytes, $\times 10^{12}$/L	9.2 ± 0.19	6.09 ± 0.41	<0.0001
Hematocrit, %	0.49 ± 0.01	0.35 ± 0.02	0.0006
Hemoglobin, g/dL	14.99 ± 0.32	10.63 ± 0.69	0.0003
Plasma biochemical parameters (at 11 weeks post-surgery)			
Sodium, mmol/L	142.3 ± 0.7	142.5 ± 1.1	0.8855
Potassium, mmol/L	3.87 ± 0.28	4.49 ± 0.12	0.0399
Calcium, mmol/L	2.42 ± 0.07	2.25 ± 0.09	0.1940
Phosphorus, mmol/L	1.72 ± 0.08	2.06 ± 0.29	0.3407
Calcium-phosphorus product	4.15 ± 0.18	4.41 ± 0.44	0.6417
Proteins, g/dL	5.53 ± 0.09	5.32 ± 0.05	0.0377
24 h urine biochemical parameters (at 9 weeks post-surgery)			
Creatinine, mmol/L	7.05 ± 0.83	2.53 ± 0.18	<0.0001
Sodium-to-creatinine ratio	7.4 ± 0.34	25.6 ± 1.27	<0.0001
Potassium-to-creatinine ratio	30.4 ± 0.6	60.5 ± 1.9	<0.0001
Calcium-to-creatinine ratio	0.11 ± 0.01	0.30 ± 0.03	0.0005
Phosphorus-to-creatinine ratio	0.45 ± 0.14	30.71 ± 1.18	<0.0001
Albumin-to-creatinine ratio, mg/mmol	275 ± 26	583 ± 105	0.011

Data are mean ± SEM.

Table 2. Serum concentration of uremic toxins assayed 11 weeks after surgery in sham-operated rats and 5/6 nephrectomized rats receiving a high-phosphate diet (5/6Nx + P).

	Sham n = 8	5/6Nx + P n = 12	*p*-Value
Indoxyl sulfate (mg/L)	0.23 ± 0.05	10.73 ± 1.98	0.0004
Paracresyl sulfate (mg/L)	0.04 ± 0.01	8.23 ± 1.19	<0.0001
Indole acetic acid (mg/L)	0.18 ± 0.02	0.45 ± 0.05	0.0002
Triethylamine oxide (mg/L)	0.11 ± 0.02	1.65 ± 0.33	0.016
Hippuric acid (mg/L)	2.11 ± 0.16	8.23 ± 1.19	0.0006

Data are mean ± SEM.

Of major importance, immunohistological examination of aortic valve sections allowed us to demonstrate the presence of hydroxyapatite crystals in 5/6Nx + P rats but not in sham-operated rats (shown in Figure 3a). In addition, there was an increased CD68+ cells in aortic valves of 5/6Nx + P rats (shown in Figure 3b), demonstrating macrophage infiltration and inflammation. Echocardiography showed classical structural and hemodynamical changes associated with aortic valve calcification, i.e., a decrease in aortic valve cusp separation and an increase in aortic valve peak flow velocity and aortic valve mean pressure gradient (shown in Figure 3c–e). In addition, alterations in cardiac systolic and diastolic function were observed with a slight but significant decrease in LV fractional shortening, ejection fraction, and cardiac output, and an increase in the E/e' ratio (shown in Figure 4a–d), while cardiac remodeling was supported from the nonsignificant increase in LV weight and the presence of LV fibrosis (shown in Figure 4e,f).

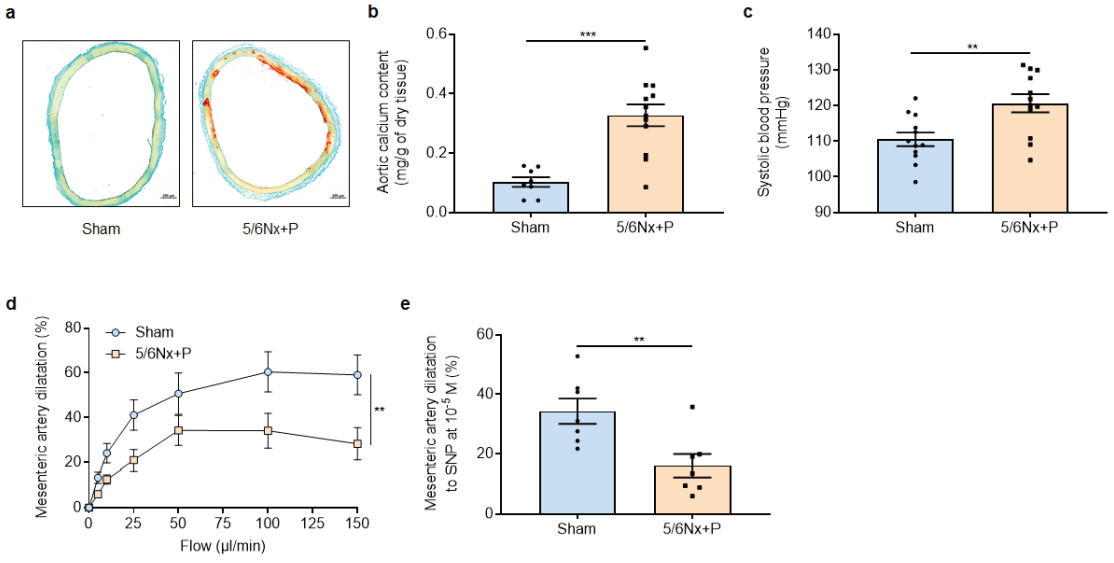

Figure 2. Representative images (**a**) and relative values of calcium content (**b**) of aortic rings stained with Alizarin red, systolic blood pressure (**c**), mesenteric artery endothelium-dependent dilatation to stepwise increase in flow (**d**), and endothelium-independent dilatation to 10^{-5} M sodium nitroprusside (**e**) obtained 10 to 11 weeks after surgery in sham-operated and 5/6 nephrectomized rats receiving a high-phosphate diet (5/6Nx + P). ** p <0.01, *** $p < 0.001$.

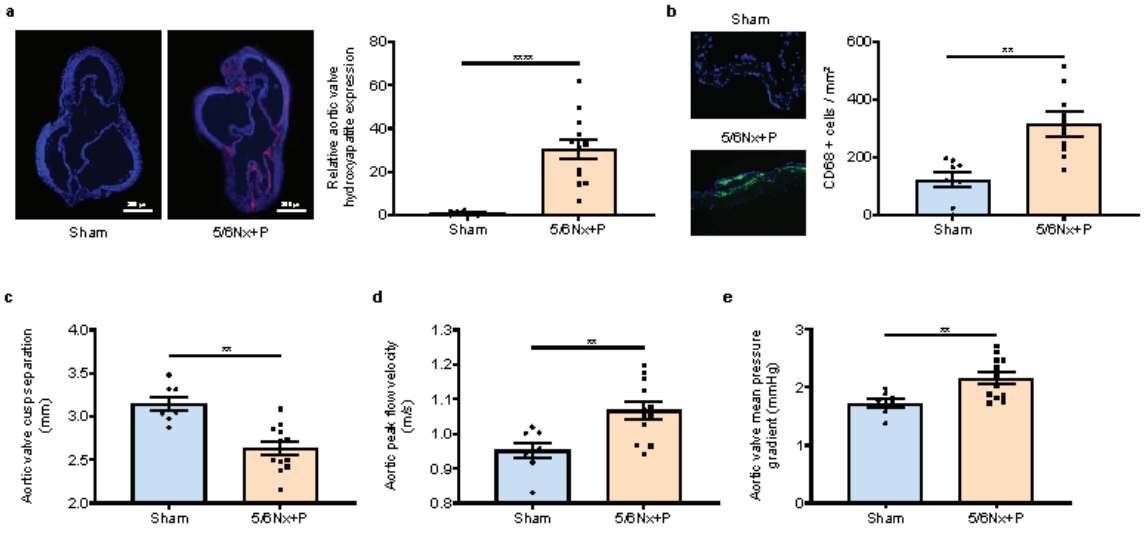

Figure 3. Representative images and relative values of hydroxyapatite expression of aortic valves stained with Osteosense 680Ex® (**a**), representative images and values of CD68+ cells per mm^2 (green and blue staining represent CD68+ cell and DAPI nuclear marker, respectively) (**b**), aortic valve cusp separation (**c**), peak flow velocity (**d**), and mean pressure gradient (**e**) obtained 10 to 11 weeks after surgery in sham-operated and 5/6 nephrectomized rats receiving a high-phosphate diet (5/6Nx + P). ** $p < 0.01$, **** $p < 0.0001$.

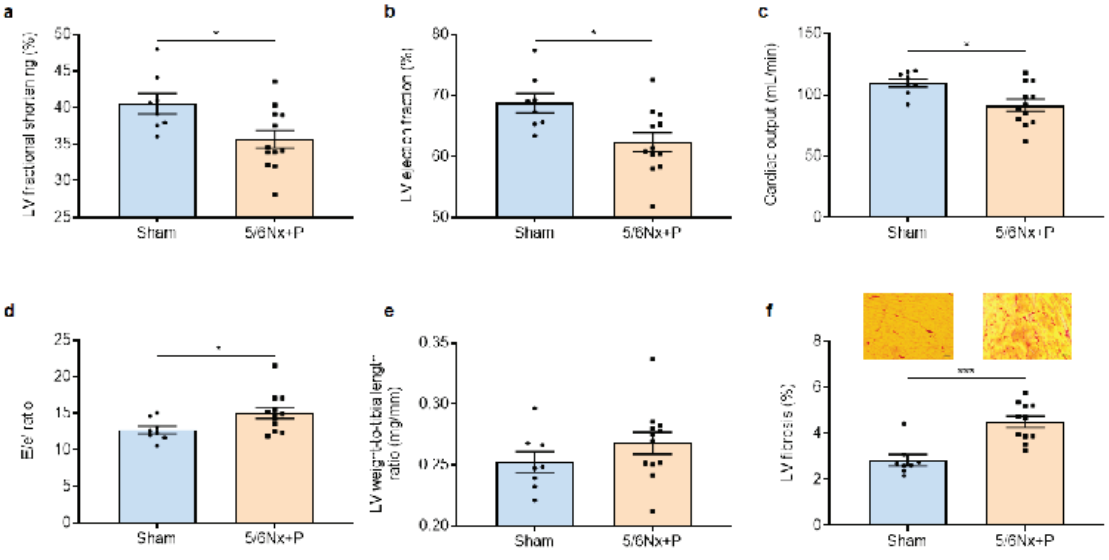

Figure 4. Left-ventricular (LV) fractional shortening (**a**), ejection fraction (**b**), cardiac output (**c**), E/e' ratio (mitral inflow E wave/e' tissue Doppler mitral annulus velocity) (**d**), LV weight-to-tibia length ratio (**e**), and representative images of Sirius red-stained LV sections and level of fibrosis (**f**) obtained 10 to 11 weeks after surgery in sham-operated and 5/6 nephrectomized rats receiving a high-phosphate diet (5/6Nx + P). * $p < 0.05$, *** $p < 0.001$.

4. Discussion

The major finding of this study is that the induction of CKD by subtotal nephrectomy in rats combined with a high-phosphate diet allowed us to promote the development of aortic valvular calcification with classical structural and hemodynamical repercussions of CAVD.

At this time, no reliable animal model for human CAVD exists, restraining the determination of the mechanisms involved in the development of aortic stenosis and the discovery of adequate therapeutic strategies. Only swine has been shown to spontaneously develop CAVD, while rabbit or murine models require genetic and/or external interventions, are time-consuming, and only a small proportion of the animals truly develop CAVD [5,6]. For this study, we decided to focus on a rat model of subtotal nephrectomy associated with a high-phosphate diet, since CKD remains a major risk factor for the development of aortic stenosis [3,4], and because one recent work demonstrates that it is associated with the development of valvular calcification [7]. We chose a phosphate diet containing 1.8% phosphorus that allowed for maintaining all other dietary nutrients at the same values than the normal 1% phosphate diet and a duration of 10 to 11 weeks since a 8-week period appeared to not be sufficient [7].

As expected from this model, 5/6Nx + P rats quickly developed all the hallmarks of CKD with decreased kidney function, a uremic state, and anemia. In this context, and despite the absence of change in the plasma calcium-phosphorus product, 5/6Nx + P rats displayed ectopic calcification at the arterial level and in the aortic valve. The latter calcifications were highlighted thanks to the use of a synthetic fluorescent bisphosphonate, which binds to hydroxyapatite crystals with high affinity [12], thus allowing us to confirm previous results obtained using sophisticated methods based on electron microscopy and X-ray diffraction [7].

As suggested in CKD patients, vascular calcification may contribute to the development of hypertension and vascular dysfunction in 5/6Nx + P rats. Importantly, we can demonstrate for the first time using echocardiography that the aortic valvular calcification

and inflammation is associated with a significant decrease in the aortic valve cusp separation and an increase in aortic valve peak flow velocity and aortic valve mean pressure gradient, which are classically used to categorize patients with CAVD. Although it is not possible to distinguish between the effects of renal dysfunction/uremic toxins and a putative early consequence of CAVD, cardiac diastolic and systolic functions were slightly altered, and cardiac remodeling was already present in 5/6Nx + P rats. The alteration in systolic function was not previously observed in this model by Wang et al. [7], but it should be kept in mind that we used older animals (18-week-old vs. 8-week-old rats) and that the severity of 5/6Nx and thus of kidney damage may vary notably depending on the extent of kidney cauterization. In our 5/6Nx + P rats, the left-ventricular remodeling (as shown by the increase in left ventricle weight and fibrosis) and both the systolic and diastolic dysfunctions of the left ventricle (as shown by the decrease in fractional shortening and the increase in the E/e' ratio, respectively) were consistent with type 4 cardiorenal syndrome [13]. This uremic cardiopathy was associated with an increase in afterload objectified in vivo by the slight but significant increase in arterial blood pressure and ex vivo by the impaired endothelial function. This led to the reduction in cardiac performance (as shown by the decrease in ejection fraction and cardiac output) that will aggravate until heart failure development.

This study demonstrates that subtotal nephrectomy combined with a high-phosphorus diet in SD rats allows us to recapitulate, over a short period, the cardiovascular hallmarks of CKD including CAVD and its hemodynamic consequences. A longer follow-up of the 5/6Nx rats on this high-phosphorus diet may be helpful to potentialize the development of aortic stenosis and cardiac alterations. However, this model already represents a promising experimental tool to study the pathophysiology of CAVD and test the impact of new therapeutic strategies targeting the valvular calcification process at an early stage, in the expected way of finally reversing the course of the disease, improving patients' health, and limiting the need for aortic valve replacement.

Author Contributions: H.M., T.L., N.P., E.B., G.F., A.D., V.B., D.G., Y.B., and J.B. acquired and analyzed the data. V.B., H.E., V.R., S.K., E.D., Y.B., and J.B. designed and supervised the project. Y.B. and J.B. drafted the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This work has been carried out in accordance with the UE Directive 2010/63/EU. The study was carried out in compliance with the ARRIVE guidelines. The protocol was approved by an Institutional Animal Care Committee (CENOMEXA C2EA-54) and the French Ministry of Education and Research (Protocol ID: APAFIS#24107).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors have no conflict of interest to declare.

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In-Hospital Mortality and Risk Prediction in Minimally Invasive Sutureless versus Conventional Aortic Valve Replacement

Giuseppe Santarpino [1,2,3,*,†], Roberto Lorusso [4], Armin Darius Peivandi [5,†], Francesco Atzeni [2], Maria Avolio [6], Angelo Maria Dell'Aquila [5,‡] and Giuseppe Speziale [7,‡]

[1] Department of Experimental and Clinical Medicine, Magna Graecia University, 88100 Catanzaro, Italy
[2] Department of Cardiac Surgery, Città di Lecce Hospital, GVM Care & Research, 73100 Lecce, Italy
[3] Department of Cardiac Surgery, Paracelsus Medical University, 90471 Nuremberg, Germany
[4] Cardio-Thoracic Surgery Department, Heart & Vascular Centre, Maastricht University Medical Hospital, 6229 HX Maastricht, The Netherlands
[5] Department of Cardiac Surgery, Münster Universität, 48143 Münster, Germany
[6] Clinical Data Management, GVM Care & Research, 00137 Rome, Italy
[7] Department of Cardiac Surgery, Anthea Hospital, GVM Care & Research, 70124 Bari, Italy
* Correspondence: santarpino@unicz.it; Tel.: +39-324-6940566; Fax: +39-0961-3694073
† These authors contributed equally to this work and should be both considered first authors.
‡ These authors contributed equally to this work and should be both considered senior authors.

Abstract: Objective. Available evidence suggests that a minimally invasive approach with the use of sutureless bioprostheses has a favorable impact on the outcome of patients undergoing aortic valve replacement (AVR). Methods. From 2010 to 2019, 2732 patients underwent conventional AVR through median sternotomy with a stented bioprosthesis (n = 2048) or minimally invasive AVR with a sutureless bioprosthesis (n = 684). Results. Using the propensity score, 206 patients in each group were matched, and the matched groups were well balanced regarding preoperative risk factors. Both unmatched and matched patients of the sutureless + minimally invasive group showed significantly shorter cross-clamp times and longer ICU stay. In-hospital mortality was the only outcome measure that was confirmed in both analyses, and was higher in the stented + conventional group (2.54% and 2.43% in unmatched and matched patients, respectively) compared with the sutureless + minimally invasive group (0.88% and 0.97% in unmatched and matched patients, respectively) (p = 0.0047 and $p < 0.0001$, respectively). No differences in postoperative pacemaker implantation were recorded in matched patients of both groups (n = 2 [1%] in the stented + conventional group vs. n = 4 [2%] in the sutureless + minimally invasive group; p = 0.41). The discrimination power of EuroSCORE II was not confirmed in the sutureless + minimally invasive group, yielding an area under the ROC curve of 0.568. Conclusions. Minimally invasive sutureless AVR has a favorable impact on the immediate outcome and is associated with significantly lower in-hospital mortality rates compared with conventional AVR, resulting in the absence of the discrimination power of EuroSCORE II for predicting AVR outcomes.

Keywords: aortic stenosis; aortic valve replacement; minimally invasive surgery; sutureless valves

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1. Introduction

Over the last several years, the optimal treatment option for aortic valve stenosis has been a subject of intense debate. The guideline indications for surgery have changed since the introduction of transcatheter aortic valve implantation (TAVI), and major interest in this issue remains given the increasing prevalence of aortic stenosis with advancing age [1].

The use of new prosthetic models and the adoption of minimally invasive approaches were initially demonstrated to be safe in patients at high or prohibitive surgical risk and have then gradually moved to lower risk patients. The new frontier of research has therefore concentrated on low-risk patients undergoing surgical aortic valve replacement (SAVR), for

whom current ESC/EACTS guidelines are somewhat "unclear": SAVR is recommended in low-risk patients (STS-PROM/EuroSCORE II <4%) or unsuitable for TAVI and operable [1]. Then, should a low-risk patient who is both operable and suitable for TAVI undergo a transcatheter procedure? Indeed, this kind of patient falls into the so-called "remaining patients" category [1], who may undergo both procedures.

Interestingly, no mentioning has been made in the current guidelines about the use of sutureless and rapid-deployment prostheses that may reduce cross-clamp and cardiopulmonary bypass times, and potentially lower perioperative complications of SAVR. As stated in the guidelines, the lack of large-scale randomized trials in this context—though a published trial does exist [2]—makes SAVR with conventional prosthetic valves the gold standard.

Similarly, minimally invasive surgery is not mentioned in the guidelines, but it is acknowledged that SAVR via full sternotomy may contribute to the development of pulmonary complications [1,3,4]. However, available evidence suggests that a minimally invasive approach has a favorable impact on the immediate outcome and is associated with lower mortality rates compared to standard sternotomy [5].

The aim of this study was to assess if minimally invasive surgery with a sutureless valve may result in a better outcome compared with full sternotomy with a stented bioprosthesis in low-risk patients and in the "remaining patients" category so as to support the Heart Team decision-making tailored to the individual patient.

2. Methods

From 2010 to 2019, data of all patients referred to nine cardiac surgery centers of the GVM Care and Research Group (Anthea Hospital, Bari, Italy; Città di Lecce Hospital, Lecce, Italy; ICLAS, Rapallo, Italy; Maria Cecilia Hospital, Cotignola, Italy; Maria Eleonora Hospital, Palermo, Italy; Maria Pia Hospital, Turin, Italy; Salus Hospital, Reggio Emilia, Italy; Santa Maria Hospital, Bari, Italy; Villa Torri Hospital, Bologna, Italy), with symptomatic severe aortic stenosis or with either steno-insufficiency with an indication for surgery after evaluation by the Heart Team, were retrieved from a single, centralized electronic data management system.

All patients aged >60 years who had undergone surgical bioprosthetic aortic valve replacement were included in this analysis.

All patients underwent conventional aortic valve replacement (AVR) through longitudinal median sternotomy or minimally invasive AVR via a ministernotomy or a right anterior minithoracotomy, according to the surgeon's experience and preference. Similarly, if a bioprosthetic aortic valve was used (usually in patients >65 years old), the choice to implant a stented or stentless valve, or a sutureless bioprosthesis, was left to the surgeon at the time of operation. However, sutureless valves were less frequently implanted in patients undergoing conventional AVR as we selected patients that were operated by experienced surgeons that had completed the learning curve.

Patients were divided into two groups based on the type of prosthetic valve used and the surgical approach they received: the stented + conventional group (n = 2048) undergoing conventional AVR with a stented bioprosthesis (the stented prostheses were either the Mosaic Ultra or the Avalus, both by Medtronic, MN, USA), and the sutureless + minimally invasive group (n = 684) undergoing minimally invasive AVR with the Perceval bioprosthesis (Corcym, Milan, Italy). A propensity score matching (PSM) analysis was used to address potential selection bias from a lack of randomization. For the matched pair samples, postoperative clinical data and hospital costs were obtained.

The study was approved by a human research ethical review board (IRB 2/2021, 19 October 2021).

The primary outcome measures were in-hospital mortality, hospital costs, cardiopulmonary bypass (CPB) and cross-clamp times, intensive care unit (ICU) and hospital stay, need for blood transfusion, and postoperative pacemaker implantation.

2.1. Surgical Approach

In the minimally invasive group, a partial J-shaped ministernotomy in the third to fourth intercostal space or a right anterior thoracotomy in the second intercostal space was performed. For both surgical approaches, CPB was established with central arterial and central or peripheral venous cannulation. Antegrade crystalloid cardioplegia was used. The stented prostheses were implanted with semi-continuous sutures or U-stitches with pledgets according to the surgeon's preference.

The implant technique of the Perceval valve has been described previously [6], along with the tips and tricks to minimize the risk for postoperative pacemaker implantation [7], which have been adopted by all surgeons involved in the study after appropriate training provided by the GVM Care and Research Group.

2.2. Statistical Analysis

Statistics were performed using MedCalc Software (MecCalc Software Ltd., Ostend, Belgium). Normality of continuous variables was tested by the Shapiro–Wilk test. Continuous variables are depicted as the median and interquartile range. Categorical variables are reported as counts and percentages. To provide a balanced data frame of patients with the same likelihood of undergoing minimally invasive sutureless AVR or conventional stented AVR, a PSM was performed according to the following: prior to matching, the influence of preoperative values (Table 1) on the decision of minimally invasive sutureless AVR was assessed by univariate logistic regression. Significant values, except EuroSCORE II (as it is a composite of preoperative values), were included in a multivariate logistic regression model for developing a propensity score. Variables not included in the model by statistical software are shown in Table 1. Statistical significance was set at $p \leq 0.05$. The propensity score was defined as the probability of receiving minimally-invasive sutureless valve replacement. After creation of the propensity score, a two-decimal digit case-control matching based on the propensity score was performed. In the matched cohort, univariate logistic regression analysis revealed no significant differences in preoperative parameters between groups as a sign of good matching (Table 1). For comparison of results in the unmatched cohort, unpaired testing was applied: continuous non-normally distributed variables were compared using a Mann–Whitney U test, and for dichotomous variables, the Chi-square test was performed. After matching, paired testing was applied as suggested by Bland and Altman [8]: continuous non-normally distributed variables were compared using the Wilcoxon test. The McNemar test was applied to dichotomous variables.

Table 1. Preoperative characteristics of the study population before and after propensity score matching.

Unmatched								
	Stented + Conventional (*n* = 2048)			**Sutureless + Minimally Invasive (*n* = 684)**			***p*-Value (uni log reg)**	***p*-Value (multi log reg)**
	Median/N	**1st Percentile**	**3rd Percentile**	**Median/N**	**1st Percentile**	**3rd Percentile**		
Male sex	981	47.90	%	250	36.55	%	0.00001	0.0233
Age, years	77	72	81	78	73	82	0.00001	0.0008
Emergency	226	11.04	%	53	7.75	%	0.0145	Not included in the model
Active endocarditis	33	1.61	%	1	0.15	%	0.0196	Not included in the model
Previous endocarditis	19	0.93	%	0	0	%	0.9844	
Creatinine preop, mg/dL	0.9	0.8	1.1	0.9	0.7	1.1	0.1492	Not included in the model
COPD	122	5.96	%	67	9.80	%	0.0002	Not included in the model
PAP >30 mmHg	627	30.62	%	411	60.09	%	0.00001	0.0315
History of syncope	198	9.67	%	13	1.90	%	0.0138	0.0806
EuroSCORE II, %	2.8	1.74	4.7	2.14	1.39	3.44	0.00001	

Table 1. *Cont.*

	Unmatched							
	Stented + Conventional (n = 2048)			Sutureless + Minimally Invasive (n = 684)			p-Value (uni log reg)	p-Value (multi log reg)
	Median/N	1st Percentile	3rd Percentile	Median/N	1st Percentile	3rd Percentile		
LVEF preop, %	55	50	60	60	55	60	0.00001	0.0005
NYHA class III or IV	1121	54.74	%	187	27.34	%	0.00001	Not included in the model
Isolated aortic valve stenosis	1671	81.59	%	588	85.96	%	0.0091	Not included in the model
	Matched							
	Stented + Conventional (n = 206)			Sutureless + Minimally Invasive (n = 206)			p-Value	
	Median/N	1st Percentile	3rd Percentile	Median/N	1st Percentile	3rd Percentile		
Male sex	77	37.38	%	67	32.52	%	0.3018	
Age, years	79	75	83	78	74	82	0.2611	
Emergency	14	6.80	%	12	5.83	%	0.6856	
Active endocarditis	4	1.94	%	0	0.00	%	0.98	
Previous endocarditis	2	0.97	%	0	0.00	%	0.9859	
Creatinine preop., mg/dL	0.9	0.7	1.1	0.8	0.7	1	0.2778	
COPD	15	7.28	%	18	8.74	%	0.5866	
PAP >30 mmHg	74	35.92	%	71	34.47	%	0.757	
History of syncope	12	5.83	%	13	6.31	%	0.8366	
EuroSCORE II, %	2.84	1.71	4.495	2.23	1.47	3.7	0.0928	
LVEF preop., %	55.5	55	60	60	55	60	0.9437	
NYHA class III or IV	108	52.43	%	126	61.17	%	0.0738	
Isolated aortic valve stenosis	171	83.01	%	173	83.98	%	0.7907	

COPD, chronic obstructive pulmonary disease; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; PAP, pulmonary artery pressure; preop, preoperatively.

Validation analysis of EuroSCORE II was conducted as described in the ABCD model by Steyerberg and Vergouwe [9]. Calibration was assessed by slope and intercept analysis. Receiver operating characteristic (ROC) curve analysis was conducted for discrimination between groups (Figure 1).

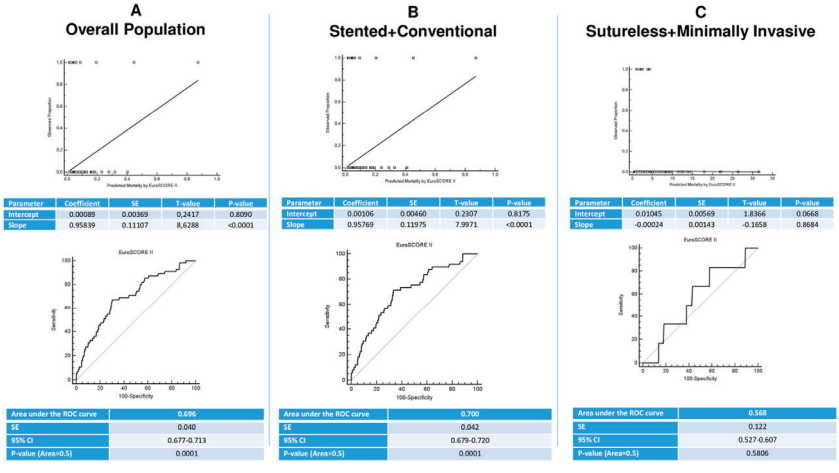

Figure 1. Calibration and discrimination of EuroSCORE II in the overall population. CI, confidence interval; SE, standard error; ROC, receiver operating characteristic.

3. Results

The preoperative characteristics of the study population are reported in Table 1. Unmatched patients of the stented + conventional group were older and at higher surgical risk compared with patients of the sutureless + minimally invasive group. The latter showed clinically pure aortic stenosis more often. The cases of concomitant surgery are those in which the aortic valve replacement has been associated with a septal myectomy.

Using the propensity score, 206 patients in each group were matched, and the matched groups were well balanced regarding preoperative risk factors (Table 1).

Postoperative results significantly differed between groups (Table 2). The matched stented + conventional group showed prolonged CPB and cross-clamp times and longer hospital stay than the sutureless + minimally invasive group, but differences were no longer present after PSM (Table 2). In contrast, both unmatched and matched patients of the sutureless + minimally invasive group showed significantly shorter cross-clamp times and longer ICU stay. Hospital costs and the need for blood transfusion were higher in unmatched stented + conventional patients, but the opposite was seen in matched patients of the same group.

Table 2. Postoperative results before and after propensity score matching.

	Unmatched						
	Stented + Conventional (*n* = 2048)			**Sutureless + Minimally Invasive (*n* = 684)**			***p*-Value**
	Median/N	**1st Percentile**	**3rd Percentile**	**Median/N**	**1st Percentile**	**3rd Percentile**	
In-hospital mortality, %	52	2.54	%	6	0.88	%	0.047
Hospital costs, €	24,181.5	20,486.6	24,675	20,896.33	20,486.6	24,675	0.4594
CPB time, min	73	58.75	88	56	43	71	<0.0001
Cross-clamp time, min	57.5	45	69	42	34	53	<0.0001
ICU stay, days	1.77	0.95	2	1.92	1.59	2.59	<0.0001
Hospital stay, days	11	8	15	10	8	13	<0.0001
Transfusions	664	32.42	%	146	21.35	%	0.065
	Matched						
	Stented + Conventional (*n* = 206)			**Sutureless + Minimally Invasive (*n* = 206)**			***p*-Value**
	Median/N	**1st Percentile**	**3rd Percentile**	**Median/N**	**1st Percentile**	**3rd Percentile**	
In-hospital mortality, %	5	2.43	%	2	0.97	%	<0.0001
Hospital costs, €	23,479.46	20,486.6	24,675	24,181.5	20,486.6	24,675.19	0.0118
CPB time, min	73	60	91.5	65	52.75	78.25	0.0627
Cross-clamp time, min	58	45	70.5	48	40	60	0.0139
ICU stay, days	1.65	0.92	1.96	1.92	1.74	2.56	<0.0001
Hospital stay, days	11	8	14	11	9	14	0.7835
Transfusions	65	31.55	%	81	39.32	%	0.0001

CBP, cardiopulmonary bypass; ICU, intensive care unit.

In-hospital mortality was the only outcome measure that was confirmed in both analyses, and was higher in the stented + conventional group (2.54% and 2.43% in unmatched and matched patients, respectively) compared with the sutureless + minimally invasive group (0.88% and 0.97% in unmatched and matched patients, respectively) ($p = 0.0047$ and $p < 0.0001$, respectively).

No differences in postoperative pacemaker implantation were recorded in matched patients of the two groups ($n = 2$ [1%] in the stented + conventional group vs. $n = 4$ [2%] in the sutureless + minimally invasive group; $p = 0.41$).

Postoperatively, in the matched population, three cerebrovascular events (1.4%; two transient ischemic attacks and one permanent neurologic deficit) were recorded in the stented + conventional group vs. one event (0.5%; one transient ischemic attack) in the sutureless + minimally invasive group ($p = 0.31$).

In the whole study population, the area under the ROC curve for EuroSCORE II was 0.696 (Figure 1A) indicating a good discrimination power. The same applies to the stented + conventional group showing an area under the ROC curve of 0.7 (Figure 1B).

On the contrary, the discrimination power of EuroSCORE II was not confirmed in the sutureless + minimally invasive group, yielding an area under the ROC curve of 0.568 (Figure 1C).

4. Discussion

Our results show that minimally invasive AVR with a sutureless bioprosthesis in patients with aortic valve stenosis is associated with significantly shorter ischemic times and lower mortality rates compared with matched patients undergoing conventional SAVR, resulting in the absence of the discrimination power of EuroSCORE II for predicting AVR outcomes. Although our study population included patients at low and intermediate surgical risk with a median EuroSCORE II of 2.23 in the selected matched cohort, this finding should be part of the Heart Team decision-making when evaluating SAVR vs. TAVI.

Even if no randomized trials have been conducted as yet on minimally invasive AVR with sutureless bioprostheses, it is difficult to understand why none of the two approaches, either in isolation or combined, have not been addressed in the recent ESC/EACTS guidelines [1].

In the prospective randomized PERSIST-AVR trial [2], sutureless valves significantly reduced surgical times and were non-inferior to stented valves with respect to major adverse cerebral and cardiovascular events at 1 year, suggesting that sutureless valves should be considered as part of a comprehensive valve program. However, patients undergoing AVR through a minithoracotomy were excluded from this study, making the assessment of the potential benefit of minimally invasive surgery with a sutureless bioprosthesis impossible.

Similarly to our study, Dalén et al. [10] analyzed early postoperative outcomes after AVR through a ministernotomy with a sutureless bioprosthesis compared with a full sternotomy with a stented bioprosthesis, showing that the former was associated with shorter CPB and aortic cross-clamp times than the latter. This is noteworthy given that the minimally invasive approach is generally considered more demanding and time-costing than open heart surgery. Additionally, patients undergoing ministernotomy received less packed red blood cells, but the technique used at that time was associated with a higher risk for postoperative pacemaker implantation. However, more recently, rates of postoperative pacemaker implantation after sutureless AVR have dramatically declined after the learning curve has been overcome [11,12]. In our study, although we recorded a twofold higher rate of postoperative pacemaker implantation in the matched sutureless + minimally invasive group compared with the stented + conventional group, it accounted for a very low percentage of patients (2%) and did not significantly differ between groups.

In addition, Pollari et al. [13] compared patients undergoing AVR with a sutureless valve vs. a stented valve showing a better short-term outcome in the sutureless group after PSM, with a total hospital cost saving of approximately 25%. However, authors' conclusions were only derived from the faster procedural time of sutureless AVR. Our multicenter study, by evaluating the effect of using a minimally invasive approach in patients referred to a variety of centers with different experience levels, allows for drawing considerations on the reproducibility of the results and on several issues related to different management protocols across the participating centers. However, despite the differences in the strategies adopted in the various centers (e.g., indications for triggering blood transfusion, length of ICU stay), mortality rates remained significantly lower in the matched sutureless + minimally invasive group. Moreover, as also intuitively expected given that patients in this group were at a lower surgical risk than unmatched patients receiving a stented valve, the EuroSCORE II lost its predictive ability also in the overall group.

One of the limitations of our article is that the patients who underwent a minimally invasive approach were operated on by a group of surgeons with a more advanced learning curve than the patients operated on with a conventional approach. However, it should be emphasized that, contrary to popular belief, the full sternotomy is still the "standard of care" in case of aortic valve replacement. Consequently, also considering the high number

of surgeons involved in our study with different levels of experience, our study reflects a real-life setting.

We also want to underline the originality of our study that, unlike other previous studies [10,14], which compared minimally invasive rapid-deployment or sutureless prostheses versus conventional approaches, we here recorded a significant difference in hospital mortality. Therefore, given the debated results, the need for a "truly" randomized trial is mandatory.

To the best of our knowledge, this is the first study demonstrating a significant impact of minimally invasive AVR using sutureless bioprostheses on in-hospital mortality. This finding was based on the data recorded by nine cardiac surgery centers and cannot be affected by the different protocols in use but rather reflects a real-life scenario.

In a prior study from our group that assessed the potential advantages of using sutureless vs. conventional prostheses for minimally invasive AVR with data collected from the same centralized electronic data management system, similar favorable outcomes were reported with a 30-day mortality of 0.7% and 2.1% in patients receiving a sutureless and a conventional prosthesis, respectively ($p = 0.076$) [15]. However, a minimally invasive strategy was used in both patient groups in this study.

In intermediate or high-risk patients, SAVR is associated with longer lasting results, and a more favorable cost-effectiveness ratio compared with TAVI is mostly attributable to the higher cost of transcatheter devices [16]. In our analysis involving low-risk patients, no differences in healthcare costs were observed between unmatched groups despite the higher cost of sutureless devices compared to conventional devices. In contrast, healthcare costs were higher in the matched sutureless group, and it would be interesting to know if costs varied according to the protocols used for blood transfusion, length of ICU, and hospital stay, etc., but this is a limitation of our study. However, either similar or higher healthcare costs are associated with a significantly lower in-hospital mortality in the sutureless + minimally invasive group. The question is whether an average cost of additional EUR 700.00 may be worth it to achieve a significant reduction in mortality in this patient subset.

Moreover, our suggested approach of minimally invasive sutureless AVR, though more expensive, is more effective and matches well with the incremental cost-effectiveness ratio calculation, resulting in a cost of EUR 479.45 per additional in-hospital life saved. However, the question of whether the gain in reduced in-hospital mortality is worth the cost remains open.

EuroSCORE II has a strong predictive ability that has recently been confirmed using data collected from our centralized database, and performs better than a parsimonious risk score [17]. In our study, in patients treated with a minimally invasive approach with a sutureless valve, the observed risk was much lower than predicted. This suggests a protective effect conferred by our strategy that should always be evaluated during preoperative planning and adopted in anatomically suitable patients (i.e., without type 0 bicuspid aortic valve).

Despite similar clinical outcomes across the different participating centers, the length of ICU stay ranged from 0.9 to 2.5 days and the length of hospital stay ranged from 12 to 16 days, where a shorter ICU stay was usually followed by a longer hospital stay. The effect of the management protocols on the length of ICU and hospital stay—which may also be observed for blood transfusion trigger/cut-off—is a clear bias and a limitation of our study, given that the centers where a longer ICU stay was recorded were those where sutureless prostheses were most often used.

5. Conclusions

In conclusion, despite the inherent limitations of our multicenter, an observational, real-life study, partially addressed by using PSM, minimally invasive sutureless AVR was associated with significantly lower in-hospital mortality rates compared with conventional surgery, and this treatment option should be considered in patients with favorable

anatomical characteristics. Further evaluation in a randomized trial combining these two procedural aspects is urgently warranted as no indications are provided in the current guidelines and information provided by independent studies is constantly growing.

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Abbreviations

AVR	aortic valve replacement
CI	confidence interval
COPD	chronic obstructive pulmonary disease
CPB	cardiopulmonary bypass
GVM	Gruppo "Villa Maria"
ICU: I	intensive care unit
LVEF	left ventricular ejection fraction
NYHA	New York Heart Association
PAP	pulmonary artery pressure
PSM	propensity score matching
ROC	receiver operating characteristic
SAVR	surgical aortic valve replacement
SE	standard error
TAVI	transcatheter aortic valve implantation

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Looking Back to Look Forward: What to Expect in a Redo Surgery for a Bioprosthesis Replacement

Ilaria Giambuzzi [1,*,†], Giorgia Bonalumi [1,*,†], Giulia Ballan [1], Pietro Messi [2], Alice Bonomi [3], Analia Maggiore [1], Giampiero Esposito [1], Michele Di Mauro [4], Francesco Alamanni [2,5] and Marco Zanobini [1]

[1] Department of Cardiac Surgery, Centro Cardiologico Monzino IRCCS, 20137 Milan, Italy
[2] I.R.C.C.S. Ospedale Galeazzi—Sant'Ambrogio, 20157 Milan, Italy
[3] Department of Biostatistics, Centro Cardiologico Monzino IRCCS, 20137, Milan, Italy
[4] Cardio-Thoracic Surgery Department, Heart & Vascular Centre, Maastricht University Medical Centre, 6229 HX Maastricht, The Netherlands
[5] Dipartimento Scienze Cliniche e di Comunità (DISCCO Department), University of Milan, 20122 Milan, Italy
* Correspondence: i.giambuzzi@hotmail.it (I.G.); giorgia.bonalumi@cardiologicomonzino.it (G.B.); Tel.: +39-0258002684 (G.B.)
† These authors contributed equally to this work.

Abstract: Redo surgeries are becoming more common because of an increased rate of bioprosthesis implantation. We performed a retrospective study on patients who underwent redo replacement of an aortic and/or mitral bioprosthesis between 2005 and 2018 to evaluate intra-hospital mortality and morbidity. Univariate analysis was performed on the propensity score variables to determine predictors of mortality. A total of 180 patients were enrolled in the study: Group A (replacement of aortic bioprosthesis) with 136 patients (75.56%) and group B (replacement of mitral bioprosthesis ± aortic bioprosthesis) with 44 patients (24.44%). NYHA class ≥ 3 and female sex were significantly more common in group B. Cardiopulmonary-bypass time and aortic cross-clamping time in group A and group B were, respectively, 154.95 ± 74.35 and 190.25 ± 77.44 ($p = 0.0005$) and 115.99 ± 53.54 and 144.91 ± 52.53 ($p = 0.0004$). Overall mortality was 8.89%. After propensity score adjustment, Group B was confirmed to have an increased risk of death (OR 3.32 CI 95% 1.02–10.88 $p < 0.0001$), gastrointestinal complications (OR 7.784 CI 95% 1.005–60.282 $p < 0.0002$) and pulmonary complications (OR 2.381 CI 95% 1.038–5.46 $p < 0.0001$). At the univariate analysis, endocarditis, cardiopulmonary-bypass and aortic cross clamping time, NYHA class ≥ 3 and urgency setting were significantly associated to death. Intra-hospital outcomes were acceptable regarding mortality and complications. Patients who need redo surgery on mitral bioprosthesis have an increased risk of post-operative pulmonary and gastrointestinal complications and mortality. Therefore the choice of mitral bioprosthesis at time of first surgery should be carefully evaluated.

Keywords: bioprosthesis replacement; redo surgery; structural valve degeneration; endocarditis

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1. Introduction

In cardiac valve surgery, the most commonly used prostheses to replace patients' diseased valves are biological ones, as opposed to mechanical ones [1,2]. Indeed, bioprostheses have several advantages: first of all, life-time anticoagulant therapy is usually deemed not necessary, even if patients might have the indications to take the anticoagulant drugs for a short period [3]. Secondly, bioprostheses are not noisy, which means that they cause less discomfort to patients. Nevertheless, there are also disadvantages linked to the use of biological valves: firstly, a smaller effective valve orifice and, secondly, the structural degeneration of the prosthesis [4]. This latter mentioned disadvantage is unavoidable at the present time and determines the need to undergo a reoperation [5,6].

The main reason for the rise in valvular reinterventions or redo surgeries is that a growing number of biological prostheses are being implanted in young patients [7], partly

due to the development of percutaneous surgeries in recent years, which may allow a future valve-in-valve procedure [8]. Indeed, biological prostheses can also be recommended for patients younger than 50 years old [9,10].

Percutaneous techniques for treating failing valvular bioprostheses are developing more and more but remain an alternative to surgery in the medium-high surgical risk group only for the aortic valve, with the TAVI technique, and for the high surgical risks associated with the more complex mitral valve. A further unknown of transcatheter valve implantation techniques is the durability of the valve bioprostheses [11] and the consequent risk in explanting a TAVR [12].

Nevertheless, redo surgery has higher mortality and morbidity when compared to first surgery [13,14].

The aim of this study is to analyze the immediate post-operative outcomes (survival and main complications) of patients who undergo redo cardiac surgery on a previously implanted bioprothesis through the assessment of a group of patients subjected to the above-mentioned surgical operation.

2. Materials and Methods

We performed a retrospective monocentric study on patients who underwent replacement of a bioprosthesis in the aortic and/or mitral position between 2005 and 2018. The study was approved by the local ethical committee (n. R1480/21-CCM 1554) with the need for consent waived given the retrospective nature of the study. Data are available upon request.

Inclusion criteria included previous surgery with implantation of biological prosthesis in aortic and/or mitral positions (in the case of double replacement, both valves were, in all cases, replaced with bioprostheses at the time of first surgery) and indication to undergo redo surgery because of malfunctioning of the valve. Exclusion criteria included only being under age.

Intraoperative data were obtained retrospectively and stored in a database.

The biological prosthesis dysfunction definition has been reviewed over the years. Aetiology for redo surgery was either endocarditis, paravalvular leak or structural valve deterioration (SVD). Our patients who underwent redo surgery because of SVD were in stage 3 of the definition proposed by Dvir Danny et al. [4].

Pulmonary complications were defined as pleural effusion and/or pneumothorax needing tube placement, pneumonia, prolonged mechanical ventilation (>48 h) and acute pulmonary insufficiency ($P/F < 100$). Gastrointestinal complications were defined as intestinal ischemia or perforation.

2.1. Diagnostic Work-Up and Surgery

In the case of elective surgery, all patients underwent echocardiographic studies to evaluate and define the aetiology of the bioprosthesis disease. In the case of endocarditis, an antibiotic therapy was also initiated. Moreover, a CT scan was performed to study adherences and the sternal relationship with the heart.

In contrast, in urgent cases, once the correct diagnosis was obtained, the CT scan might have not been performed, depending on the clinical status of the patient.

The surgery was carried out through re-sternotomy (only one patient underwent thoracotomy for replacement of mitral bioprosthesis) and cardiopulmonary bypass (CPB) was instituted either centrally or peripherally, depending on mediastinal adherences. After aortic cross clamp, the left atrium and/or aorta were opened to examine the bioprosthesis and confirm the indication (SVD, endocarditis or paravalvular leak). Subsequently, the bioprosthesis was removed and a new prosthesis was implanted. In the case of replacement of mitral and aortic bioprostheses, the mitral bioprosthesis was implanted before the aortic one. The choice of the new type of prosthesis (biological or mechanical) was discussed pre-operatively with the patient and decided upon depending on the age, comorbidities and risk of another surgery.

2.2. Statistical Analysis

Continuous variables were expressed as mean ± standard deviation, for normally distributed variables, as medians and quartiles (25–75%) for continuous variables not normally distributed and as numbers (percentages) for categorical variables. To identify differences between the two groups in terms of mean, median, or percentage, *t*-test, Wilcoxon's test, Fisher's exact test, and χ^2 were used. The multivariate logistic model was implemented to assess whether the group was a predictor of the individual endpoints (exitus, gastrointestinal complications and pulmonary complications), after adjustment for propensity score. The propensity score was estimated running a logistic model including these characteristics: preoperative ECG, NYHA class, etiology, and endocarditis; these were chosen through an epidemiological approach (i.e., those factors that in the clinician's experience can be confounders).

Finally, a univariate analysis was performed on the propensity score variables to determine predictors of mortality (as total intra-hospital death). A p-value < 0.05 was considered significant.

All analyses were performed using SAS 9.4 software.

3. Results

3.1. Pre Operative Results

A total of 180 patients underwent redo surgery between 2005 and 2018, among 8500 who underwent cardiac surgery. Patients were divided in two groups: Group A and Group B. Group A included 136 (75.56%) cases who underwent replacement of an aortic bioprosthesis. Group B included 44 (24.44%) patients who underwent replacement of a mitral valve bioprosthesis only (30 patients) or of both mitral and aortic valve bioprostheses (14 patients). Pre-operative characteristics are reported in Table 1.

Table 1. Pre operative characteristics.

	N°	Group A	Group B	*p*-Value
Total number of patients	180 (100)	136 (75.56)	44 (24.44)	
Age (years)	61 ± 14.97	60.79 ± 15.74	63.41 ± 12.08	0.316
Female sex	69 (38.33)	41 (30.15)	28 (63.64)	<0.0001
Euroscore II	10.04 ± 11.02	9.13 ± 10.47	10.96 ± 12.61	0.188
Arterial Hypertension (>140/90 mmHg)	101 (56.11)	77 (56.62)	24 (54.55)	0.809
Chronic kidney disease	124 (68.89)	93 (68.38)	31 (70.45)	0.4932
BMI	25.5 ± 3.43	25.5 ± 3.43	25.44 ± 3.5	0.003
Smoke	71 (39.44)	54 (39.71)	17 (38.64)	0.98
Cerebrovascular disease	22 (12.15)	15 (11.02)	7 (15.09)	0.07
History of AMI	13 (7.22)	8 (5.88)	5 (11.36)	0.222
Diabetes	29 (16.11)	21 (15.44)	8 (18,18)	0.59
COPD	19 (10.56)	15 (11.03)	4 (9.09)	0.716
Dyslipidemia	94 (52.22)	70 (51.47)	24 (54.55)	0.723
Peripheral vascular disease	18 (10.00)	13 (9.56)	5 (11.36)	0.7286
NYHA ≥ 3	68 (37.78)	45 (33.09)	23 (52.27)	0.025
EF	57.06 ± 10.46	57.87 ± 9.95	56.25 ± 11.91	0.4649
TDD (mm)	51.91 ± 10.41	52.5 ± 9.39	50.28 ± 8.84	0.203
Endocarditis	37 (20.55)	30 (22.06)	7 (15.91)	0.6786
Bioprosthesis degeneration	131 (72.78)	97 (71.32)	34 (77.27)	
Paravalvular leak	12 (6.67)	9 (6.61)	3 (6.82)	

Values are reported as *n* (%) if categorical variable or mean ± standard if continuous variable. BMI: body mass index; AMI: acute myocardial infarction; chronic kidney disease: kidney damage (structural/functional abnormalities, GFR < 60 mL/min/1.73 m^2, ≥months); COPD: chronic obstructive pulmonary disease; EF: ejection fraction; TDD: telediastolic diameter.

The overall mean age was 61 ± 14.97 years old. More specifically group A was 60.79 ± 15.74 years old, while that of group B was 63.41 ± 12.08.

Among the 180 patients, 111 were male and 69 female. Notably, the proportion of female sex was significantly higher in group B (63.64%) than in group A (30.15%)

($p < 0.0001$). Moreover, 68 patients (37.78%) had an NYHA class ≥ 3, 45 (33.09%) in group A and 23 (52.27%) in group B ($p = 0.025$) (Table 1). The mean telediastolic diameter was in range of normality in both groups. Aetiology for redo surgery is described in Table 1.

Out of 180 patients, 41 (22.78%) underwent surgery because of endocarditis, 125 (69.44%) had a bioprosthesis degeneration and 11 (6.11%) had a paravalvular leak. We did not observe any statistical differences between the two groups.

3.2. Intra Operative Results

Intraoperative features taken into account for this study are listed in Table 2.

Table 2. Intra operative characteristics.

	N°	Group A	Group B	*p*-Value
Time to redo (days),		2989.57 ± 2097.66	3030.84 ± 1953.00	0.4381
Emergent surgery	20 (11.11)	19 (13.96)	1 (2.27)	0.03
Aortic cross clamping time (min)	123.06 ± 54.73	115.99 ± 53.54	144.91 ± 52.53	0.0004
CPB time (min)	163.58 ± 76.64	154.95 ± 74.35	190.25 ± 77.44	0.0005
IABP, *n* (%)	6 (3.33)	3 (2.21)	3 (6.82)	0.1385
Concomitant procedures	55 (30.56)	41 (30.15)	14 (31.82)	0.836
Biological prosthesis	130 (72.22)	101 (74.26)	29 (65.91)	0.2821
Size of aortic biological prosthesis	23 (21–25)	23 (21–25)	21 (19–23)	0.825
Size of aortic mechanical prosthesis	21 (19–23)	21 (19–23)	19 (19–21)	0.76
Size of mitral biological prosthesis	27 (25–27)	//	27 (25–27)	//
Size of mitral mechanical prosthesis	27 (27–29)	//	27 (27–29)	//

Values are reported as *n* (%) if categorical variable or mean ± standard/median (IQR) if continuous variable. CPB: cardiopulmonary bypass; IABP: intra-aortic balloon pump.

The number of surgeries performed in an emergency setting were significantly higher in group A (19 patients, 13.96%), than in group B (1 patient, 2.27%) ($p = 0.03$).

Only in 11 patients (6.11%) was cardiopulmonary bypass instituted through femoral vessels. In all other cases, a central cannulation was preferred.

Clamping time was also statistically different between the two groups: 115.99 ± 53.54 min in group A versus 144.91 ± 52.53 in group B ($p = 0.0004$). Lastly, cardiopulmonary bypass (CPB) time was observed to be higher in group B (190.25 ± 77.44) than in group A (154.95 ± 74.35) ($p = 0.0005$).

Of the whole considered population, 30.56% underwent concomitant procedures (including tricuspid valve repair and/or aorto-coronary bypass) but no difference between the two groups was noticed.

3.3. Post-Operative Results

Overall, 16 patients (8.89%) out of 180 died after surgery, 8 in group B (18.18%) and 8 in group A (5.88%). Hence, the mortality in group B was statistically higher than in group A ($p = 0.0001$). Among causes of death, seven patients (43.75%) died because of multiorgan failure, one patient (6.25%) because of intestinal ischemia, one patient (6.25%) because of intractable haemorrhage in the operating room and seven patients (43.75%) because of intractable cardiac failure. Moreover, among the 16 deceased patients, 12 (75%) underwent surgery because of endocarditis and 6 (37.56%) were operated on in an urgency setting. Anyway, even without including in the analysis patients who had endocarditis (37, 20.5%), mortality was similar. Indeed, on a total of 143 patients, there were 9 deaths (6.29%), in group A 106 with 5 deaths (4.71%) and in group B 37 patients with 4 deaths (10.81%).

Post-operative complications are listed in Table 3.

Table 3. Post-operative complications.

	N°	Group A	Group B	*p*-Value
Death	16 (8.89)	8 (5.88)	8 (18.18)	0.0001
Neurological complications	14 (7.77)	9 (6.6)	5 (11.36)	0.676
IMA	1 (0.56)	1 (0.74)	0 (0.00)	0.568
ECMO	2 (1.11)	0 (0.00)	2 (4.55)	0,9371
Pulmonary complications	45 (25)	28 (20.59)	17 (38.64)	0.0001
Arrhythmias	48 (26.67)	35 (25.74)	13 (29.54)	0.619
PM implant	17 (9.44)	14 (10.29)	3 (6.82)	0.493
Gastrointestinal complications	5 (2.78)	2 (1.47)	3 (6.82)	0.0002
Acute kidney disease, *n* (%)	85 (47.22)	65 (47.79)	20 (45.45)	0.068
Re-exploration for bleeding	20 (11.11)	17 (12.50)	3 (6.82)	0.2972
LOS ICU (days)	4.62 ± 8.83	4.04 ± 5.33	6.43 ± 15.05	0.0919
LOS (days)	14.23 ± 13.63	13.98 ± 11.61	15.02 ± 18.52	0.7685
Prolonged LOS (>14 days)	51 (28.33)	40 (29.41)	11 (25.00)	0.471

Values are reported as *n* (%) if categorical variable or mean ± standard if continuous variable. Acute kidney disease: KDIGO parameters; AMI: acute myocardial infarction; arrhythmias: atrial fibrillation, atrial flutter, ventricular tachycardia; ECMO: extracorporeal membrane oxygenation; PM: pacemaker; LOS: length of stay; ICU: intensive care unit.

Pulmonary complications affected 45 patients (25%) in total; group B reported a higher percentage of these complications (38.64%) than group A (20.59%) ($p = 0.0001$). GI complications were also higher in group B: 6.82% vs. 1.47% ($p = 0.0002$). After the propensity score adjustment, it was confirmed that patients in group B had a significantly higher risk of mortality, gastrointestinal and pulmonary complications (Table 4).

Table 4. Propensity score adjustment.

Variable	OR	95% Confidence Interval	*p* Value
Exitus	3.32	1.02–10.88	<0.0001
Gastrointestinal complications	7.784	1.005–60.282	<0.0002
Pulmonary complications	2.381	1.038–5.46	<0.0001

A univariate analysis was then performed to evaluate potential risk factors for mortality in our whole population. All analyzed variables are listed in Table 5.

Table 5. Univariate regression analysis.

Variable	OR	95% Confidence Interval		*p* Value
Female sex	0.592	0.211	1.659	0.3188
Pre-operative rhythm	0.663	0.308	1.427	0.2931
NYHA ≥ 3	0.363	0.183	0.721	0.0038
CPB time	0.987	0.98	0.993	<0.0001
Aortic cross clamping time	0.987	0.979	0.994	0.0009
Urgency setting	0.156	0.049	0.492	<0.0001
Endocarditis	0.288	0.099	0.834	0.0218

CPB: cardiopulmonary bypass.

4. Discussion

In the last few years of valvular surgery, bioprostheses are being implanted more commonly. Therefore, surgeons are facing many redo surgeries to replace a failing biological prosthesis. In order to evaluate the impact of REDO surgery for replacement of biological prosthesis on intra-hospital outcomes, we performed a retrospective study on patients who underwent replacement of aortic and/or mitral biological prosthesis. The aetiologies taken into account were either SVD, endocarditis or paravalvular leak. The overall mortality

was 8.89%. Moreover, the results showed that replacement of mitral bioprosthesis was an independent risk factor for death, gastrointestinal and pulmonary complications.

Our mortality was in line with already published data, being between 7.3% and 10.9% [15–19]. Among predictors of mortality, our univariate analysis found that CPB time, aortic cross-clamping time, NYHA $\geq$ 3, urgency setting and endocarditis were significant predictive factors. Sex was not a significant predictor, which is in line with literature, which shows conflicting results. Indeed, Vogt et al. [20] and Pansini et al. [18] found a higher mortality in females, while Akins et al. [17] reported an increased mortality in males. Longer CPB time and aortic cross-clamping times are known risk factors for worse surgical outcomes, as are urgency and endocarditis. Indeed, 43.7% of exitus was present in patients who underwent surgery for endocarditis, which is also in line with previously published studies [17,19]. Moreover, a higher NYHA class is known to be a risk factor for mortality [7,19,20].

Of interest, replacement of mitral bioprosthesis was a risk factor for death, gastrointestinal and pulmonary complications. A similar result was reported by Jones et al. and Lytle et al. [13–19], whose studies demonstrated that patients undergoing mitral valve replacement have a higher risk of mortality than patients undergoing aortic valve replacement, mainly due to post-operative acute myocardial infarction, rupture of the left ventricle and arrhythmias. Nevertheless, the causes of death in our patients in group B were multi-organ failure and cardiogenic shock. The increased mortality in patients who underwent a replacement of a mitral bioprosthesis might have both surgical and clinical reasons. First of all, surgical access to the mitral valve requires a deeper lysis of adherences and an increased manipulation of the heart. Furthermore, patients are usually more frail. Indeed, patients in group B were more prone to have an NYHA $\geq$ 3, indicating a worse underlying clinical state [21].

Replacement of a mitral valve bioprosthesis resulted in an increased risk of gastrointestinal complications. In the literature [17,22–24], causes are mainly related to low cardiac output syndrome, post-operative arrhythmias (no difference in our pool of patients), use of noradrenaline and intra-aortic balloon pump (which had a higher incidence in group B in our study) and CPB time (significantly higher in group B). Moreover, Balsam et al. [21] showed a correlation between NYHA $\geq$ 3 and gastrointestinal complications, in line with our results. Pulmonary complications might also be related to the worse clinical picture of the patients in group B because of the underlying pathology. Mitral pathology already causes an altered lung function, and it could be exacerbated in a redo surgery.

Our study shows that a redo surgery to change a biological prosthesis is not risk-free. Despite advent of valve-in-valve procedures, their use might not be indicated in all cases, such as endocarditis, risk of patient–prosthesis mismatch, high risk of embolization and left ventricle tract obstruction. Moreover, long term results are still lacking. Therefore, the role of the heart team during the first surgery becomes pivotal in assess, as much as possible, the possibility of permitting a future valve-in-valve procedure and the risk of a future re-intervention.

The most important limitation of our study is its retrospective nature. Moreover, it covers a long span of time, therefore developments in surgical techniques have been made during it. It lacks a long term follow up, even though this was not the primary objective of the study, it could give a deeper insight to the results of the surgery.

5. Conclusions

In our experience, redo surgery for replacement of mitral bioprosthesis carries an increased risk of mortality and serious complications (gastrointestinal and pulmonary). Therefore, the choice of biological prosthesis at the time of first surgery must be carefully evaluated, and the anatomical criteria for a future percutaneous mitral valve- in-valve procedure might be assessed at the time of the first surgery, in order to assess the possibility of performing a minimally invasive treatment for a future prosthesis dysfunction. Nevertheless, the patients should be aware that, in case of a bioprosthesis dysfunction

needing a traditional open surgery (which might anyway be the only option, especially in case of endocarditis), mitral bioprosthesis replacement carries an higher risk in terms of mortality, gastrointestinal and pulmonary complications when compared to other standard redo valvular surgery.

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Global Burden and Improvement Gap of Non-Rheumatic Calcific Aortic Valve Disease: 1990–2019 Findings from Global Burden of Disease Study 2019

Chengzhi Yang [1,†], Haobo Xu [2,†], Ruofei Jia [1], Zening Jin [1], Changlin Zhang [3,*] and Jiansong Yuan [2,*]

[1] Department of Cardiology and Macrovascular Diseases, Beijing Tiantan Hospital, Capital Medical University, Beijing 100070, China
[2] Department of Cardiology, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100037, China
[3] Department of Cardiology, the Second Affiliated Hospital of Dalian Medical University, Dalian 116023, China
* Correspondence: changlin_zhang@dmu.edu.cn (C.Z.); yuanjiansong@fuwaihospital.org (J.Y.)
† These authors contributed equally to this manuscript.

Abstract: The aim of this study was to explore the most updated changing trends of non-rheumatic calcific aortic valve disease (nrCAVD) and reveal possible improvements. We analyzed the age-standardized rates (ASRs) of prevalence, incidence, disability-adjusted life-years (DALYs), and mortality trends of nrCAVD from 1990 to 2019 using data from the Global Burden of Disease (GBD) study 2019. The relations between ASRs and socio-demographic index (SDI) were analyzed with Pearson's correlation coefficients. Decomposition and frontier analysis were employed to reveal the contribution proportion of influence factors and regions where improvement can be achieved. In 2019, there were 9.40 million (95% uncertainty interval (UI): 8.07 to 10.89 million) individuals with nrCAVD globally. From 1990 to 2019, the prevalence rate of nrCAVD increased by 155.47% (95% IU: 141.66% to 171.7%), with the largest increase observed in the middle SDI region (821.11%, 95% UI: 709.87% to 944.23%). Globally, there were no significant changes in the mortality rate of nrCAVD (0.37%, 95% UI: −8.85% to 7.99%). The global DALYs decreased by 10.97% (95% UI: −17.94% to −3.46%). The population attributable fraction (PAF) of high systolic blood pressure increased in the population aged 15–49 years, while it declined slightly in population aged 50+ years. Population growth was the main contributing factor to the increased DALYs across the globe (74.73%), while aging was the driving force in the high-SDI region (80.27%). The Netherlands, Finland, Luxembourg, Germany, and Norway could reduce DALY rates of nrCAVD using their socio-demographic resources. According to these results, we revealed that the burden of nrCAVD increased markedly from 1990 to 2019 in high-SDI and high-middle-SDI regions. There was a downward trend in the mortality due to nrCAVD since 2013, which is possibly owing to profound advances in transcatheter aortic valve replacement. Some countries may reduce burdens of nrCAVD using their socio-demographic resources.

Keywords: non-rheumatic calcific aortic valve disease (nrCAVD); global burden of disease; prevalence; disability-adjusted life-years (DALYs); socio-demographic index; contribution factor; improvement

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1. Introduction

The 20th anniversary of transcatheter aortic valve replacement (TAVR) has seen profound advances since its appearance in 2002, owing to dramatically increased patients with aortic valve stenosis [1]. Several studies have demonstrated that non-rheumatic calcific aortic valve disease (nrCAVD) is the main pathological basis of aortic valve stenosis nowadays [2,3]. The past three decades witnessed rapid economic and social developments, with increased burden of non-communicable diseases. As one of these disease burdens, the prevalence of nrCAVD increased markedly and carried a significant risk of mortality

and morbidity in the global population [4–6]. In a substudy of the SCOT-HEART multicenter randomized controlled trial, the authors showed that aortic valve calcification was observed in 241 (14%) of the 1769 participants aged 58 ± 9 years [7]. In addition, a meta-analysis including 22 studies found that the prevalence of aortic sclerosis ranged from 9% in a study where the mean age was 54 years to 42% in a study where the mean age was 81 years [8]. However, the studies concerning the burden of nrCAVD across the globe are very limited. Although Simon Yadgir et al. reported the general global changes of non-rheumatic valvular diseases from 1990 to 2017 [9], the global burden of nrCAVD was not investigated in detail, particularly the influence factors of nrCAVD epidemiology and potential improvement gap.

In the present study, we sought to explore the most updated changing trends of nrCAVD with the Global Burden of Disease (GBD) study 2019 data. Particularly, we used decomposition and frontier analysis to reveal the contribution proportion of influence factors and regions where improvement can be possibly achieved.

2. Methods

2.1. Data Sources and Case Definitions

We used data from the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) 2019, which was designed to provide a comprehensive assessment of health loss due to diseases, causes of death, and risk factors at the global, regional, and national levels from 1990 to 2019. The non-rheumatic calcific aortic valve disease (nrCAVD) was defined based on the International Classification of Diseases Ninth Revision (ICD-9) and Tenth Revision (ICD-10) codes.

2.2. Socio-Demographic Index (SDI)

The socio-demographic index (SDI) was employed to determine the relationship between the development status of a region or country and the burden of nrCAVD. The SDI is a composite indicator of social development levels that correlate with health outcomes. It is calculated from national-level income per capita, educational attainment in the population $\geq$ 15 years old, and women fertility rate under 25 years old. The SDI ranges from 0 (minimum development) to 1 (maximum development), and 204 countries and territories were categorized into five groups based on SDI quintiles: low SDI, low-middle SDI, middle SDI, high-middle SDI, and high SDI.

2.3. Estimation of Prevalence, Incidence, and Disability-Adjusted Life-Years

The prevalence and incidence of nrCAVD was estimated using DisMod-MR 2.1, a Bayesian meta-regression tool by which GBD 2019 collected and analyzed data from hospital discharges, publications, and household surveys. The disability-adjusted life-years (DALYs) is calculated by adding the years of life lost (YLLs) due to premature death and the years lost due to disability (YLDs) in the population.

The age-standardized rates (ASRs) of prevalence, incidence, deaths, and DALYs were generated by summarizing the products of the age-specific rates and corresponding number of persons in the same age subgroup of the GBD 2019 standard population, and then dividing by the sum of the standard population weights. The changes of ASRs between 1990 and 2019 were shown with percentage changes. Uncertainty intervals (UIs) were calculated from 1000 draws for each quantity. The 95% UIs were defined as the 25th and 975th ordered draw of the uncertainty distribution. For all analysis, a 95% UI and 95% confidence intervals (CIs) were considered to be statistically significant when zero was excluded.

2.4. Risk Factors for DALYs

The inclusion criteria of attributable risk factors were previous evidence of causation with nrCAVD and availability of exposure data in GBD 2019. The final risk factors included were high systolic blood pressure, diet high in sodium, and lead exposure.

2.5. Statistical Analysis

Spearman's rank order correlation was used to measure the strength and direction of the association between the SDI and age-standardized rates. The change in the SDI between 1990 and 2019 (the ratio of the index in 2019 to the index in 1990), and the average annual percentage change (AAPC) of ASR during 1990–2019 was calculated. To assess the magnitude and direction of trends in the ASR of nrCAVD over time, we used JoinPoint software (Version 4.7.0.0) to calculate the AAPC and the corresponding 95% confidence interval (CI) by joinpoint regression analysis. By comparing AAPC with 0, we ascertained whether the variation trend in different sections is statistically significant.

The decomposition analysis is an analytic approach to identify the additive contribution of the effect of the differences in factors in 2 populations (such as the population in 1990 and the population in 2019) on the difference in their overall value. The decomposition of nrCAVD DALYs by the different causes allows the quantification of the contribution of each cause to the overall nrCAVD DALYs. We first used the decomposition methodology of Das Gupta to decompose nrCAVD DALYs by population age structure, population growth, and epidemiologic changes (DALYs rate) [10,11]. The number of DALYs at each location was obtained from the following formula:

$$\mathrm{DALY}_{\mathrm{ay,\ py,\ ey}} = \sum_{i=1}^{20} (\mathrm{a}_{\mathrm{i,\ y}} * \mathrm{p}_{\mathrm{y}} * \mathrm{e}_{\mathrm{i,\ y}}) \quad (1)$$

where $\mathrm{DALY}_{\mathrm{ay,\ py,\ ey}}$ represent DALYs based on the factors of age structure, population, and DALYs rate for specific year y; $\mathrm{a}_{\mathrm{i,\ y}}$ represents the proportion of population for the age category i of the 20 age categories in given year y; p_{y} represents the total population in given year y; and $\mathrm{e}_{\mathrm{i,\ y}}$ represents DALYs rate given age category i in year y. The contribution of each factor to the change in DALYs from 1990 to 2019 was defined by the effect of one factor changing while the other factors were held constant.

For example, the effect of age structure was calculated as follows:

$$\begin{aligned}&[(\mathrm{DALY}_{\mathrm{a2019,\ p1990,\ e1990}} + \mathrm{DALY}_{\mathrm{a2019,\ p2019,\ e2019}})/3 + (\mathrm{DALY}_{\mathrm{a2019,\ p1990,\ e2019}} + \mathrm{DALY}_{\mathrm{a2019,\ p2019,\ e1990}})/6] - \\ &[(\mathrm{DALY}_{\mathrm{a1990,\ p2019,\ e2019}} + \mathrm{DALY}_{\mathrm{a1990,\ p1990,\ e1990}})/3 + (\mathrm{DALY}_{\mathrm{a1990,\ p2019,\ e1990}} + \mathrm{DALY}_{\mathrm{a1990,\ p1990,\ e2019}})/6]\end{aligned} \quad (2)$$

In order to evaluate the relationship between the burden of nrCAVD and socio-demographic development, we applied a frontier analysis as a quantitative methodology to identify the lowest potentially achievable age-standardized DALYs rate on the basis of development status as measured by the socio-demographic index (SDI). In this method, data envelopment analysis (DEA) would be used for frontier analysis. The frontier can be produced from several deterministic algorithms, such as free disposability hull (FDH), variable returns to scale (VRS), and so on. We used the FDH in our analysis. To incorporate stochastic variation into the frontier, we used 1000 bootstrapped samples of the data. Each bootstrap includes a subset of locations produced by randomly sampling with replacement from all countries in the Global Burden of Disease study. This accounts for autocorrelation of locations over time. In each bootstrapped sample, the following procedure was performed: Firstly, remove one data point at a time to generate a DEA and identify if the removed point is a superefficient point (outlier). Then, put the data point back, remove the second data point to generate DEA, and examine if it is the superefficient point (outlier). In this method, the superefficient point is defined as the unit whose number of age-standardized DALY rate is less than the frontier line at each SDI value calculated after removing the unit. After all the points are examined and removed all superefficient points (outliers), we then generate the frontier using DEA with FDH algorithm. We repeat this step for 1000 iteration bootstrapping, and the mean nrCAVD DALYs frontier at each SDI value from the bootstrapped samples was computed for each country at each year. Finally, LOESS regression with a local polynomial degree of 1 and span of 0.2 was then developed to generate a smoothed frontier [12,13]. To understand the relationship of age-standardized nrCAVD DALY rates vis-à-vis the frontier in 2019, we calculated the effective difference (the absolute distance from the frontier) using 2019 SDI and age-standardized nrCAVD

DALYs rate data point for each country or territory. Countries or territories with lower DALYs than the frontiers were assigned a zero distance.

The detailed description of the frontier analysis is described in the Supplementary Method. All the data analyses were conducted with the R program (Version 4.0.4, R core team).

3. Results

3.1. Prevalence, Incidence, DALYs, and Mortality of nrCAVD

Globally, the past 30 years witnessed a marked upward trend in the prevalence of nrCAVD (Table 1, Figure 1; average annual percent change (AAPC) = 3.36 (95% CI: 2.77 to 3.95)). The estimated prevalence cases of nrCAVD increased from 1.73 million (95% UI: 1.43 million to 2.07 million) in 1990 to 9.40 million (95% UI: 8.07 million to 10.89 million) in 2019. At the same time, the age-standardized prevalence rate per 100 000 population (ASPR) increased from 45.54 (95% UI: 37.61 to 54.67) to 116.34 (95% UI: 100.39 to 134.5), indicating a 155.47% (95% UI: 141.66% to 171.7%) augment in ASPR. By sex, the ASPR of nrCAVD in males was higher than that in females (133.38 vs. 99.86 in 2019), whereas the AAPC was comparable between females and males. In terms of age, global prevalence rates of nrCAVD increased with age before 95 years old in both 1990 and 2019. The ASPR of nrCAVD was very low before 30 years old and declined after 95 years old (Figure 2). Regionally, the highest ASPR of nrCAVD was reported in Australasia (649.5, 95% UI: 552.0 to 772.74) and Central Europe (608.31, 95% UI: 517.94 to 713.51) in 2019. In the past 30 years, the largest increase in ASPR was reported in East Asia (1920.64%, 95% UI: 1545.86% to 2360.13%). With respect to countries, the highest ASPR change of nrCAVD was observed in Denmark (2859.7%, 95% UI: 2329.24% to 3473.71%), and the largest number of individuals with nrCAVD was noted in China (867,917, 95% UI: 687,948 to 1,064,921) in 2019 (Supplementary Table S1).

Table 1. The prevalence of cases and age-standardized prevalence rate of non-rheumatic calcific aortic valve disease in 1990 and 2019, and their temporal trends from 1990 to 2019.

	1990		2019		1990–2019	1990–2019
	Cases No. (95% UI)	ASPR (per 100,000) No. (95% UI)	Cases No. (95% UI)	ASPR (per 100,000) No. (95% UI)	ASPR Percentage Change (95% UI)	AAPC No. (95% CI)
Global	1,732,989 (1,431,469 to 2,074,809)	45.54 (37.61 to 54.67)	9,404,078 (8,079,604 to 10,889,727)	116.34 (100.39 to 134.5)	155.47 (141.66 to 171.7)	3.36 (2.77 to 3.95)
Female	838,493 (690,259 to 1,016,936)	40.28 (32.99 to 48.82)	4,376,817 (3,771,235 to 5,082,805)	99.86 (86.1 to 115.88)	147.89 (134.1 to 165.7)	3.31 (2.86 to 3.75)
Male	894,496 (741,593 to 1,067,498)	51.19 (42.68 to 60.91)	5,027,261 (4,276,877 to 5,861,586)	133.38 (113.79 to 154.58)	160.54 (146.7 to 176.16)	3.38 (2.68 to 4.08)
High SDI	1,324,934 (1,090,000 to 1,602,157)	126.83 (104.61 to 152.72)	5,095,444 (4,402,067 to 5,933,379)	273.52 (237.08 to 315.22)	115.66 (102.05 to 130.27)	2.79 (2.18 to 3.41)
High-middle SDI	364,934 (300,689 to 436,285)	33.9 (27.98 to 40.44)	3,569,820 (3,002,415 to 4,203,730)	174.53 (147.33 to 204.54)	414.83 (391.56 to 443.21)	5.89 (5.55 to 6.23)
Middle SDI	30,505 (24,085 to 37,764)	2.82 (2.23 to 3.49)	658,545 (529,926 to 800,781)	25.93 (20.92 to 31.47)	821.11 (709.87 to 944.23)	8.04 (7.89 to 8.19)
Low-middle SDI	9361 (7345 to 11,713)	1.45 (1.15 to 1.79)	67,258 (54,355 to 81,974)	4.78 (3.86 to 5.82)	229.82 (201.99 to 259.51)	4.16 (4.08 to 4.24)
Low SDI	2930 (2295 to 3719)	1.12 (0.88 to 1.38)	9675 (7605 to 12,135)	1.67 (1.34 to 2.07)	49.94 (44.14 to 56.39)	1.39 (1.34 to 1.44)
High-income Asia Pacific	469,556 (383,483 to 566,437)	233.42 (191.41 to 280.25)	1,715,700 (1,450,883 to 2,042,127)	408.4 (348.93 to 479.89)	74.96 (63.35 to 86.36)	1.94 (1.8 to 2.08)

Table 1. *Cont.*

	1990		2019		1990–2019	1990–2019
	Cases No. (95% UI)	**ASPR (per 100,000) No. (95% UI)**	**Cases No. (95% UI)**	**ASPR (per 100,000) No. (95% UI)**	**ASPR Percentage Change (95% UI)**	**AAPC No. (95% CI)**
High-income North America	687,664 (557,975 to 840,921)	191.35 (155.87 to 232.93)	1,492,891 (1,305,203 to 1,727,501)	244.39 (214.46 to 279.6)	27.72 (13.83 to 44.93)	1.86 (0.81 to 2.92)
Western Europe	218,212 (177,736 to 268,116)	37.65 (30.75 to 45.97)	1,862,787 (1,577,560 to 2,209,085)	204.84 (174.62 to 240.59)	444 (398.78 to 494.86)	6.38 (5.73 to 7.03)
Australasia	9877 (8177 to 11,826)	41.77 (34.78 to 49.88)	320,825 (272,234 to 381,057)	649.5 (552 to 772.74)	1454.86 (1236.35 to 1697.07)	9.97 (9.85 to 10.08)
Andean Latin America	831 (661 to 1026)	3.77 (3.02 to 4.63)	33,352 (28,251 to 39,016)	59.04 (50.07 to 69.05)	1465.67 (1241.46 to 1733.05)	10.1 (9.49 to 10.72)
Tropical Latin America	8034 (6435 to 9814)	7.79 (6.23 to 9.54)	58,601 (47,232 to 72,054)	23.73 (19.16 to 29.01)	204.51 (180.25 to 234)	3.81 (3.7 to 3.92)
Central Latin America	6996 (5589 to 8531)	7.98 (6.44 to 9.71)	76,957 (63,860 to 91,384)	31.82 (26.45 to 37.75)	298.86 (261.69 to 344.2)	5.06 (4.14 to 6)
Southern Latin America	5497 (4391 to 6936)	11.87 (9.53 to 14.91)	101,893 (87,124 to 120,797)	122.72 (104.78 to 145.67)	933.61 (766.84 to 1118.16)	8.22 (8.07 to 8.38)
Caribbean	3359 (2751 to 4009)	12.61 (10.3 to 15.1)	45,467 (37,694 to 54,849)	87.51 (72.62 to 105.56)	594.1 (504.42 to 696.1)	7.07 (6.73 to 7.41)
Central Europe	154,665 (128,343 to 184,574)	104.18 (86.62 to 123.47)	1,260,558 (1,067,648 to 1,479,616)	608.31 (517.94 to 713.51)	483.91 (434.98 to 537.45)	6.63 (6.24 to 7.02)
Eastern Europe	121,662 (97,120 to 148,877)	43.32 (34.79 to 52.76)	1,328,687 (1,065,696 to 1,605,206)	395.8 (319.64 to 477)	813.6 (752.33 to 886.02)	8.12 (7.89 to 8.34)
Central Asia	3767 (2956 to 4713)	7.92 (6.22 to 9.83)	41,060 (33,495 to 48,810)	53.22 (43.89 to 62.92)	572.13 (489.66 to 669.91)	6.82 (6.7 to 6.93)
North Africa and Middle East	9696 (7638 to 11,898)	4.92 (3.9 to 6.04)	54,300 (43,226 to 66,785)	10.83 (8.67 to 13.35)	120.05 (104.74 to 135.33)	3.01 (2.55 to 3.47)
South Asia	8332 (6398 to 10,638)	1.34 (1.04 to 1.67)	30,188 (23,678 to 37,633)	2.03 (1.6 to 2.52)	51.55 (45.4 to 58.54)	1.44 (1.4 to 1.48)
Southeast Asia	1785 (1337 to 2357)	0.7 (0.53 to 0.91)	23,986 (18,739 to 30,273)	4.06 (3.21 to 5.09)	480.45 (408.41 to 566.35)	6.17 (6.05 to 6.29)
East Asia	18,272 (13,682 to 24,325)	2.1 (1.57 to 2.74)	891,018 (707,255 to 1,093,313)	42.41 (33.87 to 51.68)	1920.64 (1545.86 to 2360.13)	11.07 (10.58 to 11.57)
Oceania	110 (89 to 136)	4.3 (3.49 to 5.36)	1130 (904 to 1374)	18.61 (14.95 to 22.75)	332.86 (271.32 to 396.31)	4.7 (4.17 to 5.24)
Western Sub-Saharan Africa	1300 (1004 to 1627)	1.27 (0.99 to 1.58)	3870 (3002 to 4876)	1.65 (1.3 to 2.04)	29.76 (25.16 to 34.98)	0.85 (0.76 to 0.94)
Eastern Sub-Saharan Africa	763 (589 to 963)	0.93 (0.73 to 1.15)	2602 (2004 to 3271)	1.4 (1.1 to 1.74)	51.64 (43.67 to 61.1)	1.47 (1.41 to 1.53)
Central Sub-Saharan Africa	245 (189 to 315)	1 (0.79 to 1.26)	796 (621 to 1012)	1.36 (1.08 to 1.69)	36.9 (28.71 to 46.73)	1.12 (1.06 to 1.18)
Southern Sub-Saharan Africa	2365 (1845 to 2936)	8.15 (6.36 to 10.13)	57409 (44,278 to 73,386)	94.86 (72.97 to 120.7)	1064.03 (877.48 to 1280.35)	9.02 (8.62 to 9.41)

AAPC, average annual percentage change; ASPR, age-standardized prevalence rate; SDI, socio-demographic index; UI, uncertainty interval; CI, confidence interval.

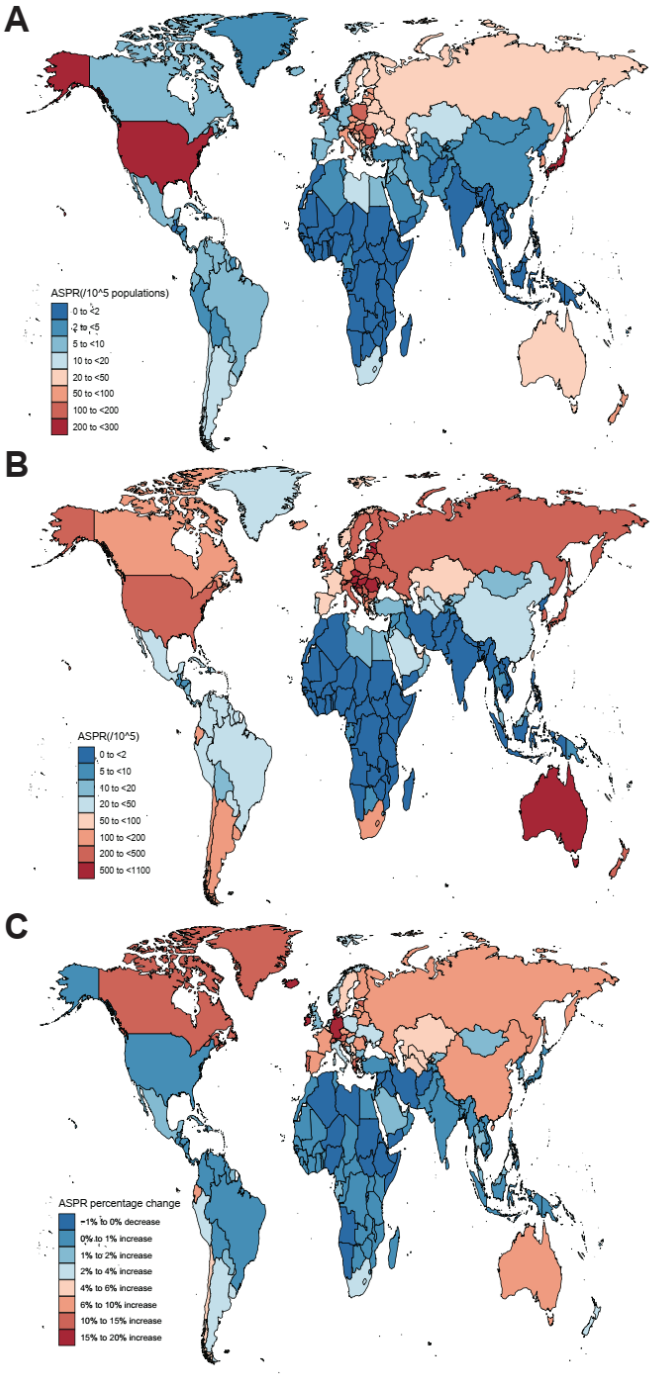

Figure 1. The global age-standardized prevalence rate (ASPR) of non-rheumatic calcific aortic valve disease (nrCAVD) in 204 countries and territories. (**A**) The ASPR of nrCAVD in 1990. (**B**) The ASPR of nrCAVD in 2019. (**C**) The relative change in ASPR of nrCAVD between 1990 and 2019.

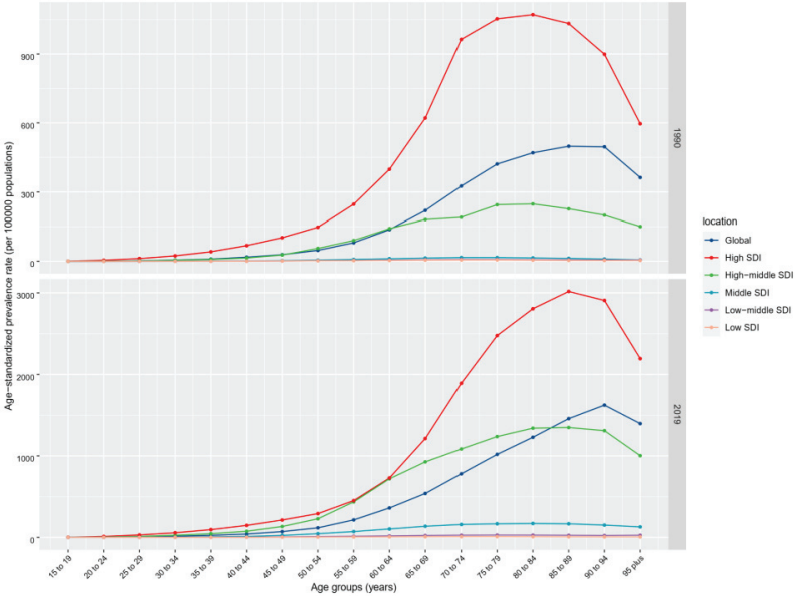

Figure 2. The ASPR of nrCAVD by age group in 1990 and 2017. ASPR, age-standardized prevalence rate; nrCAVD, non-rheumatic calcific aortic valve disease.

The global incidence number of nrCAVD was 589,638 (95% UI: 512,895 to 677,062) in 2019, with a 350.72% increase from 130,822 (95% UI: 110,701 to 156,022) in 1990 (Supplementary Table S2). Besides, the age-standardized incidence rates per 100,000 population (ASIR) increased from 3.25 (95% UI: 2.76 to 3.86) to 7.13 (95% UI: 6.22 to 8.15), showing an increase of 119.24 (95% UI: 108.72 to 131.69). In 2019, the highest ASIR was reported in Australasia (44.39, 95% UI: 38.08 to 51.79) and Central Europe (33.16, 95% UI: 28.29 to 38.67). The greatest increase in ASIR was observed in Australasia (698.05%, 95% UI: 597.68% to 830.16%), followed by Eastern Europe (678.78%, 95% UI: 631.44% to 732.11%) and East Asia (665.55%, 95% UI: 544.2% to 807.08%).

As shown in Supplementary Table S3, the global age-standardized DALYs rate (ASDR) of nrCAVD decreased from 26.85 (95% UI: 24.07 to 30.31) per 100,000 person-years in 1990 to 23.9 (95% UI: 21.1 to 26.55) per 100,000 person-years in 2019, showing a reduction of 10.97% (95% UI: −17.94% to −3.46%). The ASDR of nrCAVD varied substantially among different regions. In 2019, the highest ASDR of nrCAVD was reported in Western Europe (51.94, 95% UI: 45.69 to 56.95) while the lowest ASDR was reported in East Asia (4.36, 95% UI: 3.59 to 5.18). Over the past 30 years, the largest decrease in DALYs was found in high-income Asia Pacific (−35.91%, 95% UI: −45.57 to −27.93) while the greatest increase was shown in Eastern Europe (169.8%, 95% UI: 114.91% to 265.33%).

Globally, there were no significant changes in the age-standardized mortality rate (ASMR) per 100,000 population of nrCAVD between 1990 and 2019 (0.37%, 95% UI: −8.85% to 7.99%; Supplementary Table S4), while the deaths caused by nrCAVD increased from 53,298 (95% UI: 47,760 to 59,731) to 126,827 (95% UI: 105,603 to 141,390). In detail, there were two periods when the ASMR of nrCAVD increased, 1990 to 1993 and 2003 to 2013. On the other hand, the ASMR decreased in two periods, 1994 to 2002 and 2013 to 2019.

Stratified by SDI regions and sex, the prevalence, incidence, DALYs, and mortality of nrCAVD correlated positively with SDI for both men and women (Supplementary Figures S1 and S2). Consistently, the highest ASPR, ASIR, ASDR, and ASMR were seen in the high-SDI region. At the same time, the middle-SDI region witnessed the greatest increase in ASPR (821.11%, 95% UI: 709.87% to 944.23%), and the high-middle-SDI region saw the greatest increase in ASIR (298.74%, 95% UI: 283.11% to 316.0%).

3.2. Risk Factors for nrCAVD

We studied the trends of population attributable fraction (PAF) of three exposure risk factors for DALYs of nrCAVD, high systolic blood pressure (HSBP), diet high in sodium, and lead exposure over the past 30 years. Globally, the age-standardized PAF for HSBP accounted for 38.02% (95% UI 30.27% to 46.42%) in 1990, and 33.62% (95% UI 25.98% to 42.46%; Supplementary Figure S3) in 2019 of DALYs. The PAF of HSBP declined slightly in population aged 50+ years, while it increased in those aged 15–49 years. Besides, the PAF of diet high in sodium and lead exposure remained at a low level and relatively constant from 1990 to 2019. The PAF trends for these risk factors were similar between men and women at all age groups.

Stratified by SDI regions, the PAF of the three risk factors declined in all age groups in the high-SDI region, except for diet high in sodium in those aged 15–49 years (Supplementary Figure S4). For the high-middle SDI region, the trends of PAF for the three risk factors were similar to the global trends. For other SDI regions, the PAF of HSBP increased in all age groups, while the PAF of diet high in sodium declined in most age groups.

3.3. Decomposition of nrCAVD

In order to identify the contribution of population growth, aging, and epidemiological changes to the trends of nrCAVD epidemiology over the past three decades, we conducted a decomposition analysis of raw DALYs by age structure, population growth, and epidemiological changes (referring to age- and population-standardized mortality rates). The raw DALYs of nrCAVD were increased in all SDI quintiles, and the increase extent of DALYs was positively related to SDI values. Globally, the population growth contributed 74.73% to the increased burden of nrCAVD DALYs from 1990 and 2019, while the epidemiological changes contributed 25.49% to the decreased burden of nrCAVD DALYs (Figure 3, Supplementary Table S5). The contribution of aging to raw DALYs was highest in the high-SDI region (80.27%); decreased to 42.56% in the high-middle region, 41.2% in the middle region, 23.77% in the low-middle region; and was counterproductive in the low-SDI region (−1.87%). Over the same period, the contribution of population growth showed a nearly contrary trend to that of aging. Of note, although the contribution of epidemiological changes was decreased in most GBD regions, it increased in Central Europe and Eastern Europe, where it contributed 69.83% and 82.86% to the burden of nrCAVD DALYs, respectively.

3.4. Frontier Analysis of nrCAVD

Furthermore, we performed a frontier analysis based on age-standardized DALY rates (ASDR) of nrCAVD and SDI values to explore the possible improvement in the ASDR that is potentially realized given a nation's development status. The countries and territories with lowest ASDR (optimal performers) based on corresponding SDI values were indicated by the frontier line. The distance from the frontier line is the gap between a country's observed and potentially achievable DALYs. This distance is called effective difference. The effective difference could be potentially reduced or eliminated taking the country's socio-demographic resources. Globally, the effective difference for a given SDI tended to be larger and more variable as SDI increased (Figure 4, Supplementary Table S6). The top 10 countries with the largest effective difference were Cyprus, Slovenia, Hungary, Uruguay, Bermuda, New Zealand, Greenland, Belgium, Austria, and Argentina. Besides, there were some countries and territories with high SDI (>0.85) but relatively high effective difference, such as Netherlands, Finland, Luxembourg, Germany, and Norway. By contrast, several countries with low SDI (<0.5) showed small effective difference, including Chad, Niger, Mali, Cambodia, and Laos.

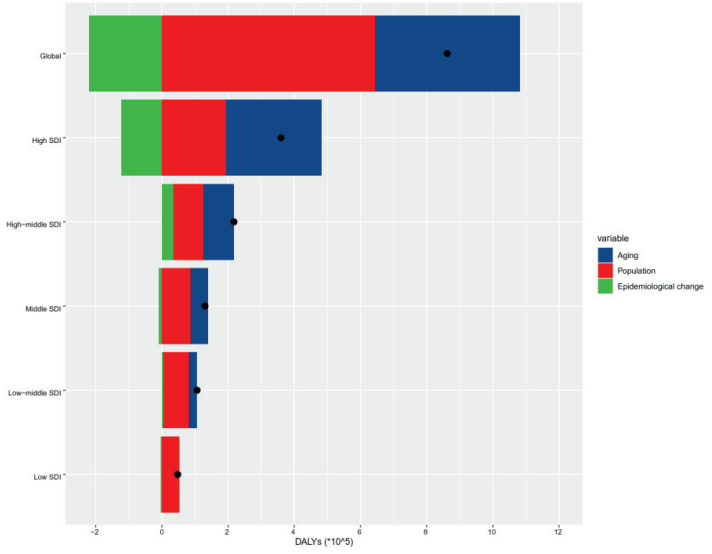

Figure 3. Changes in nrCAVD DALYs according to population-level determinants of population growth, aging, and epidemiological change from 1990 to 2019 at the global level and by SDI quintile. The black dot represents the overall value of change contributed by all three components. For each component, the magnitude of a positive value indicates a corresponding increase in nrCAVD DALYs attributed to the component, and the magnitude of a negative value indicates a corresponding decrease in nrCAVD DALYs attributed to the related component. nrCAVD, non-rheumatic calcific aortic valve disease; SDI, socio-demographic index.

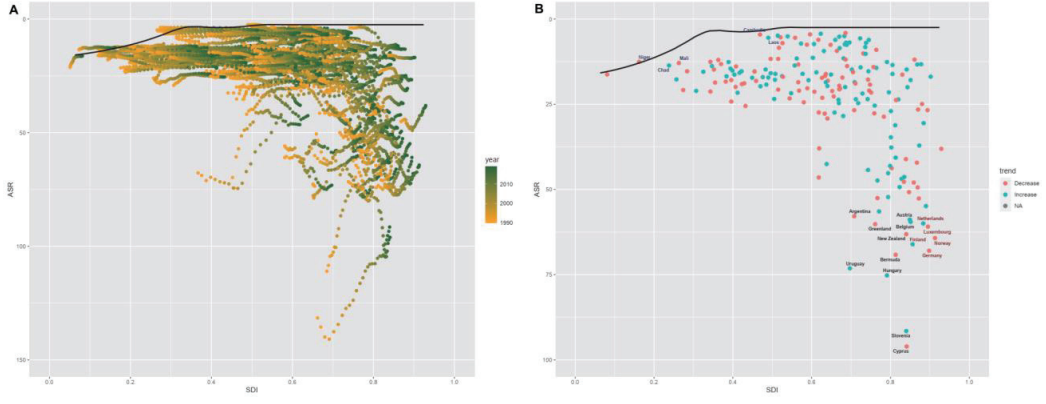

Figure 4. (**A**) Frontier analysis based on SDI and age-standardized nrCAVD DALYs rate from 1990 to 2019. The frontier is delineated in solid black color; countries and territories are represented as dots. (**B**) Frontier analysis based on SDI and age-standardized nrCAVD DALYs rate in 2019. The top 10 countries with the largest effective difference (largest nrCAVD DALYs gap from the frontier) are labeled in black; examples of frontier countries with low SDI (<0.5) and low effective difference are labeled in blue (e.g., Chad, Niger, Mali, Cambodia, and Laos); and examples of countries and territories with high SDI (>0.85) and relatively high effective difference for their level of development are labeled in red (e.g., Netherlands, Finland, Luxembourg, Germany, and Norway). Red dots indicate an increase in age-standardized nrCAVD DALYs rate from 1990 to 2019; blue dots indicate a decrease in age-standardized nrCAVD DALYs rate between 1990 and 2019. DALYs, disability-adjusted life years; SDI, socio-demographic index; nrCAVD, non-rheumatic calcific aortic valve disease.

4. Discussion

The non-rheumatic calcific aortic valve disease (nrCAVD) has attracted more and more attention over the past 30 years. In this study, based on the most updated Global Burden of Disease (GBD) study data, we found that the global age-standardized prevalence and incidence rate of nrCAVD increased markedly from 1990 to 2019, while there were no overt changes in the age-standardized DALYs and mortality due to nrCAVD. The population attributable fraction (PAF) of high systolic blood pressure declined slightly in population aged 50+ years, while it increased in those aged 15–49 years. The population growth was the main contributing factor to the increased DALYs across the globe, while aging was the driving force in the high-SDI region. Netherlands, Finland, Luxembourg, Germany, and Norway could reduce DALY rates of nrCAVD using their socio-demographic resources.

Previous studies showed that the prevalence of nrCAVD increased obviously in some countries or regions over the past three decades [8,14,15]. In line with prior reports, we found that both prevalence cases and age-standardized prevalence rate (ASPR) of nrCAVD increased markedly from 1990 to 2019. In addition, the incidence of nrCAVD also increased over the same period. There are several risk factors related to nrCAVD pathogenesis [16]. First, it is well established that aging is an important stimulus to nrCAVD [17]. A recent study of 944 participants aged ≥65 years reported that overall prevalence of nrCAVD was 22.0%, with 16.7% in individuals aged 65~69 years and 67.0% in individuals aged ≥85 years [18]. We also found that the prevalence of nrCAVD sharply surges with advancing age. Interestingly, the global ASPR declined in individuals aged 95+ years, a phenomenon not reported previously. A possible explanation for this turning relation between ASPR and age may be that the nrCAVD and concurrent diseases with shared risk factors would lead to early death before 95 years old. The turning point of ASPR according to age came later in 2019 than that in 1990, which may be attributable to population aging and healthcare progress.

Many studies have demonstrated that hypertension is one of the causes of nrCAVD and can result in about a 20% increase in risk of nrCAVD [19–21]. In a prospective, observational study of 101 participants with mild or moderate aortic stenosis, Lionel Tastet et al. found that patients with systolic hypertension had much faster aortic valve calcification progression compared with those without systolic hypertension during a 2-year follow-up [22]. In addition, in a cohort study of 5.4 million UK participants, the authors reported that each 20 mmHg increment in systolic blood pressure (SBP) was associated with a 41% higher risk of aortic stenosis and a 38% higher risk of aortic regurgitation, and these associations were stronger in younger participants [23]. In the current study, we observed that DALYs of nrCAVD attributable to high SBP increased in most regions from 1990 to 2019, except for the high-SDI region. Noteworthily, the risk factor attribution of high SBP increased in individuals <50 years old, while it decreased in those ≥50 years old. These two findings are coordinated because population aging in the high-SDI region is more severe than that in developing regions. The effect of high SBP on nrCAVD in terms of age in our study is in line with what was found in the UK cohort [24]. Our findings suggest that better management of high SBP may help to reduce the burden of nrCAVD in developing countries, especially for younger individuals. Of note, the causal relationship between excessive sodium consumption and increased blood pressure has long been demonstrated. However, our study showed that diet high in sodium had little effect on the disease burden of nrCAVD. Hence, the present study indicates that sodium is not an independent risk factor for nrCAVD.

It is well established that the most profound factors promoting epidemiologic transition over the past 30 years are population growth and aging, which lead to a dramatically increased burden of non-communicable diseases [4]. It is very important to identify the contribution of each risk factor in different countries so as to reduce the burden of nrCAVD and improve health care. The present studied revealed that population growth was the main contribution to the increased burden of nrCAVD DALYs globally, and this contribution proportion was inversely related to the SDI value. By contrast, aging contributed more

to the increased burden of nrCAVD in regions with higher SDI value. Our findings indicate that improvements in access to healthcare would reduce the burden of nrCAVD.

Mortality due to nrCAVD is being modified by advances in transcatheter aortic valve replacement (TAVR) over the past ten years [17,24]. A recent study based on the US National Center for Health Statistics reported that age-adjusted mortality rate (ADMR) of aortic stenosis increased before 2013 and declined after that [25]. Interestingly, we found that the global ADMR of nrCAVD also increased from 2003 to 2013 and declined after 2013. This ADMR trend of nrCAVD was especially obvious in the high-SDI region. The decrease in ADMR since 2013 occurred at a time when the number of TAVR procedures increased markedly, which suggested the reduced mortality of nrCAVD may be related to TAVR therapy.

The increased burden of non-communicable diseases was accompanied by social, economic, and medical developments [4,26]. Therefore, it is not surprising to find that the prevalence, incidence, mortality, and DALYs of nrCAVD were positively related to SDI values in our study. The prevalence and incidence of nrCAVD are much higher in the high-SDI and high-middle-SDI regions than those in other regions. Notably, the case number of nrCAVD is very large in some developing countries. For instance, China has the most cases of nrCAVD, which increased about fifty fold over the past 30 years. A similar trend might occur in India in the following years. Therefore, it is urgent for these regions to take actions to reduce the burden of nrCAVD. Using frontier analysis, our study suggests that some developed countries, such as Germany and Norway, can also reduce the burden of nrCAVD if appropriate measures are taken in the future. Considering the rapid advances are being achieved in TAVI, they may play an important role in reducing the burden of nrCAVD. It may be necessary for these regions to introduce and popularize TAVI therapy in future.

There were several limitations in the present study. First, although the DisMod-MR 2.1 could control potential biases in the data of GBD 2019, the inadequate quality and quantity of input data from some regions may have adverse effects on the accuracy of estimates. Second, different methods of diagnosis may result in differences in the incidence and prevalence of nrCAVD. The transthoracic echocardiography (TEE) is less sensitive than cardiac computed tomography in detecting nrCAVD, whereas nrCAVD is diagnosed by TEE in most cases [27,28]. Third, symptomatic and asymptomatic nrCAVD, and aortic stenosis and aortic regurgitation were mixed in the GBD 2019 database. Subtyping characteristics of nrCAVD are needed to identify the burden of nrCAVD in detail in the future.

5. Conclusions

The prevalence and incidence of nrCAVD increased markedly from 1990 to 2019, particularly in high-SDI and high-middle-SDI regions. Population growth was the main contributing factor to the increased burden of nrCAVD globally, while aging played the leading role in the high-SDI region. Better management of high SBP or young individuals may help to reduce burden of nrCAVD. There was a downward trend in the mortality due to nrCAVD since 2013, which is possibly owing to profound advances in transcatheter aortic valve replacement. Some countries with a high SDI value, such as Germany and Norway, may reduce burdens of nrCAVD using their socio-demographic resources.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm11226733/s1, Figure S1: The age-standardized prevalence rate, incidence rate, mortality and disability-adjusted life-years of nrCAVD versus SDI quintile for men and women, 1990–2019. nrCAVD, non-rheumatic calcific aortic valve disease; SDI, socio-demographic index; Figure S2: The ASPR of nrCAVD versus SDI quintile for men and women by region, 1990–2019. The black line, a LOWESS smoother, shows the expected value only on the SDI values of the global regions between 1990 and 2019. ASPR, age-standardized prevalence rate; LOWESS, locally weighted scatterplot smoothing; nrCAVD, non-rheumatic calcific aortic valve disease; SDI, socio-demographic index; Figure S3. Percentage contributions of major risk factors attributed for DALYs of nrCAVD stratified by age and sex. DALYs, disability-adjusted life-years; nrCAVD, non-rheumatic calcific

aortic valve disease; Figure S4: Percentage contributions of major risk factors attributed for DALYs of nrCAVD stratified by SDI quintile and age group. DALYs, disability-adjusted life-years; nrCAVD, non-rheumatic calcific aortic valve disease; SDI, socio-demographic index; Table S1: The prevalence cases and age-standardised prevalence rate of non-rheumatic calcific aortic valve disease in 1990 and 2019, and their temporal trends from 1990 to 2019 in 204 countries and territories; Table S2: The incidence cases and age-standardized incidence rate of non-rheumatic calcific aortic valve disease in 1990 and 2019 and its temporal trends from 1990 to 2019; Table S3: The DALYs and age-standardized DALYs rate of non-rheumatic calcific aortic valve disease in 1990 and 2019 and its temporal trends from 1990 to 2019; Table S4: The death cases and age-standardized mortality rate of cardiomyopathy between 1990 and 2019 and its temporal trends from 1990 to 2019; Table S5: Changes in DALYs number according to population-level determinants from 1990 to 2019; Table S6: Frontier DALYs and effective difference by country or territory [29].

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Core Lab Adjudication of the ACURATE *neo2* Hemodynamic Performance Using Computed-Tomography-Corrected Left Ventricular Outflow Tract Area

Ahmed Elkoumy [1,2], Andreas Rück [3], Won-Keun Kim [4], Mohamed Abdel-Wahab [5], Mahmoud Abdelshafy [1], Ole De Backer [6], Hesham Elzomor [1], Christian Hengstenberg [7], Sameh K. Mohamed [1], Nawzad Saleh [3], Shahram Arsang-Jang [1,8], Henrik Bjursten [9], Andrew Simpkin [10], Christopher U. Meduri [3] and Osama Soliman [1,8,*]

[1] Discipline of Cardiology, Saolta Group, Galway University Hospital, Health Service Executive and CORRIB Core Lab, University of Galway, H91 V4AY Galway, Ireland
[2] Islamic Center of Cardiology, Al-Azhar University, Nasr City, Cairo 11651, Egypt
[3] Department of Cardiology, Karolinska University Hospital, 17176 Stockholm, Sweden
[4] Department of Cardiology and Cardiac Surgery, Kerckhoff Heart and Lung Centre, 61231 Bad Nauheim, Germany
[5] Department of Cardiology, Heart Center Leipzig at University of Leipzig, 04289 Leipzig, Germany
[6] The Heart Center—Rigshospitalet, 2100 Copenhagen, Denmark
[7] Division of Cardiology, Department of Internal Medicine II, Medical University of Vienna, 1090 Vienna, Austria
[8] CÚRAM, SFI Research Centre for Medical Devices, H91 W2TY Galway, Ireland
[9] Department of Cardiothoracic Surgery, Anesthesia and Intensive Care, Skane University Hospital, Lund University, 22185 Lund, Sweden
[10] Data Science Institute, University of Galway, H91 TK33 Galway, Ireland
* Correspondence: osama.soliman@universityofgalway.ie; Tel.: +353-91-493-781

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Abstract: (1) Background: Hemodynamic assessment of prosthetic heart valves using conventional 2D transthoracic Echocardiography-Doppler (2D-TTE) has limitations. Of those, left ventricular outflow tract (LVOT) area measurement is one of the major limitations of the continuity equation, which assumes a circular LVOT. (2) Methods: This study comprised 258 patients with severe aortic stenosis (AS), who were treated with the ACURATE *neo2*. The LVOT area and its dependent Doppler-derived parameters, including effective orifice area (EOA) and stroke volume (SV), in addition to their indexed values, were calculated from post-TAVI 2D-TTE. In addition, the 3D-LVOT area from pre-procedural MDCT scans was obtained and used to calculate corrected Doppler-derived parameters. The incidence rates of prosthesis patient mismatch (PPM) were compared between the 2D-TTE and MDCT-based methods (3) Results: The main results show that the 2D-TTE measured LVOT is significantly smaller than 3D-MDCT (350.4 $\pm$ 62.04 mm^2 vs. 405.22 $\pm$ 81.32 mm^2) (95% Credible interval (CrI) of differences: -55.15, -36.09), which resulted in smaller EOA (2.25 $\pm$ 0.59 vs. 2.58 $\pm$ 0.63 cm^2) (Beta = -0.642 (95%CrI of differences: -0.85, -0.43), and lower SV (73.88 $\pm$ 21.41 vs. 84.47 $\pm$ 22.66 mL), (Beta = -7.29 (95% CrI: -14.45, -0.14)), respectively. PPM incidence appears more frequent with 2D-TTE- than 3D-MDCT-corrected measurements (based on the EOAi) 8.52% vs. 2.32%, respectively. In addition, significant differences regarding the EOA among the three valve sizes (S, M and L) were seen only with the MDCT, but not on 2D-TTE. (4) Conclusions: The corrected continuity equation by combining the 3D-LVOT area from MDCT with the TTE Doppler parameters might provide a more accurate assessment of hemodynamic parameters and PPM diagnosis in patients treated with TAVI. The ACURATE *neo2* THV has a large EOA and low incidence of PPM using the 3D-corrected LVOT area than on 2D-TTE. These findings need further confirmation on long-term follow-up and in other studies.

Keywords: aortic stenosis; ACURATE *neo2*; left ventricular outflow tract; hemodynamic performance; computed tomography; echocardiography; prosthesis patient mismatch

1. Introduction

Transcatheter aortic valve implantation (TAVI) is a recommended interventional strategy in selected patients with severe aortic stenosis (AS) [1,2]. Transcatheter heart valve (THV) hemodynamic performance requires detailed and accurate assessment at multiple time points to determine the device's success and detect prosthetic dysfunction. The first post-TAVI hemodynamic measurements are typically obtained before or early after hospital discharge, and are considered a baseline characterization of the implanted device (fingerprint) [3,4]; further follow-up can be compared with this baseline. Transthoracic echocardiography (TTE) is the gold standard imaging modality to assess the hemodynamic performance of THVs by measuring the peak velocity, transvalvular pressure gradients, effective orifice area (EOA), stroke volume, dimensionless velocity index (DVI) and the residual post-TAVI aortic regurgitation [3–6]. Several of these hemodynamic parameters are dependent on measuring the left ventricular outflow tract area (LVOT) area.

LVOT area calculation by the monoplane 2D-TTE is based on the measurement of a single diameter in mid-systole from the parasternal long-axis view, which resembles the small anteroposterior diameter and assumes a circular shape of the LVOT. Error in area estimation due to geometric assumptions will lead to an erroneous calculation of all derived parameters such as EOA and SV [7,8]. Accordingly, the accurate assessment of hemodynamic parameters mandates reducing or eliminating errors in LVOT area measurements.

The ACURATE *neo2* (Boston Scientific, Marlborough, MA, USA), is a new generation of self-expandable, supra-annular THV, with favorable outcomes, including a significant reduction in the incidence of residual regurgitation in comparison to the earlier iteration ACURATE *neo* [9,10]. Data on the hemodynamic performance of the ACURATE *neo2* are scarce and based only on 2D-TTE.

We hypothesized that measurements of LVOT area in a 3D fashion, from gated multiphase reconstructed multidetector computed tomography (MDCT) scans, could result in different hemodynamic performance values, and thus, reclassification of the 2D-TEE-derived ACURATE *neo2* THV outcome. In this study, we sought to assess the LVOT-dependent hemodynamic parameters such as EOA, stroke volume and their indexed values through the multimodal imaging approach, combining the more accurate 3D-MDCT-derived LVOT area and the 2D-TTE Doppler values. Reporting of these corrected values may give a true estimation of the hemodynamic performance of the ACURATE *neo2*.

2. Materials and Methods

This is a Core-Lab-led post hoc analysis of the Early neo2 Registry, a multi-center investigator-initiated European Registry of the first patients treated with the ACURATE *neo2* THV Prosthesis in Europe after market approval (NCT04810195). This study is a retrospective analysis of patients with severe native AS or malfunctioning bioprosthetic surgical aortic valves who underwent TAVI with the ACURATE *neo2* THV. We included patients with available pre-TAVI multi-phase MDCT scans and the comprehensive 2D-TTE assessment within seven days from the index procedure. All TTE and MDCT analyses were performed by three well-experienced senior cardiologists (AE, HE and MA).

The primary outcomes were the changes in hemodynamic classification of prosthesis patient mismatch (PPM) and the differences in the LVOT-dependent parameters (EOA, SV and their indexed values) between the 2D-TTE-derived continuity equation (CE) and the 3D-corrected CE by combining the MDCT-derived 3D-LVOT area and 2D-TTE Doppler measurements. The rate of PPM (moderate and severe PPM) between the two methods and the rate of reclassifications were reported.

2.1. Definition of Prosthesis Patient Mismatch (PPM)

Prosthesis patient mismatch (PPM) was identified as an EOA smaller than expected or the normal value, which led to inadequate cardiac output to meet the patient's body demands, despite a normally functioning device without structural abnormality [2]. The

indexed EOA (EOAi) is the main parameter used to assess the PPM according to the guideline's recommendations [2,4].

- For patients with BMI < 30 kg/cm^2; PPM is:
  - Hemodynamically insignificant if the indexed EOA is >0.85 cm^2/m^2.
  - Moderate if between 0.66 and 0.85 cm^2/m^2.
  - Severe if ≤0.65 cm^2/m^2.
- For obese patients with BMI ≥ 30 kg/m^2; PPM is:
  - Hemodynamically insignificant if the indexed EOA is >0.70 cm^2/m^2.
  - Moderate if between 0.56 and 0.70 cm^2/m^2.
  - Severe if ≤0.55 cm^2/m^2.

2.2. Echocardiography

A comprehensive 2D-TTE assessment of post-TAVI patients was performed before hospital discharge or within seven days from the index procedure according to the recommended guidelines for evaluating prosthetic heart valves [3,6]. Echocardiographic analyses were performed according to the Core Lab Standard Operating Procedures (SOP) based on the most recent guidelines recommendations [3–6], using a dedicated workstation (TOMTEC ARENA, TOMTEC Imaging Systems GmbH, Unterschlessheim, Germany). Velocity time integral (VTI) of blood flow across the THV (VTI_{AV}) was measured from the Continuous-wave Doppler (CWD) and that of the LVOT (VTI_{LVOT}) was measured from the pulsed-wave Doppler (PWD) of LVOT. Both measurements were obtained from the 3- or the 5-chamber apical views, if appropriate. The sample volume for the VTI_{LVOT} was typically positioned at the LV edge of the THV in systole. As recommended by guidelines, the external LVOT diameter was measured from the parasternal long-axis view in zoomed view below the prosthetic stent (inflow level) in mid-systole (Figure 1). LVOT area was calculated automatically with the formula ($A = \pi r^2$) and used to calculate the EOA using the CE in addition to the calculation of SV across the LVOT using the flow equation ($Flow_{LVOT}$ (SV_{LVOT}) = LVOT area × VTI_{LVOT}). All hemodynamic parameters values were indexed to the patient's body surface area.

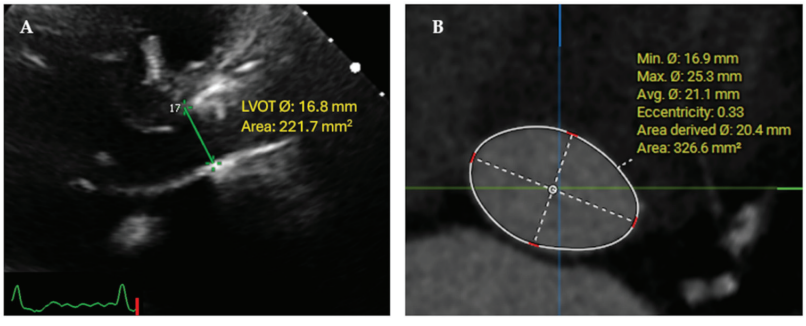

Figure 1. LVOT measurements: (**A**) 2D-TTE LVOT diameter measurements from the parasternal long axis zoomed view in mid-systole, (**B**) MDCT multiplanar reconstruction of the LVOT (5 mm below the annular plane, in mid-systolic phase 30%) with the minimum (anteroposterior) diameter and the maximum (medio-lateral) diameter with measured 3D-LVOT area, with a larger area calculated from the MDCT measured minimum and maximum diameters with eccentricity index of 0.33.

2.3. Multidetector Computerized Tomography (MDCT)

Pre-TAVI MDCT scans acquisition was performed according to each center's protocol. Offline 3D multiplanar reconstruction and comprehensive analysis were performed according to the Core Lab SOP in accordance with the Society of Cardiac Computed Tomography (SCCT) guidelines [11]. The LVOT was measured at 5 mm below and perpendicular to

the predefined native aortic annulus level from contrast enhanced MDCT scans, using a dedicated workstation (3mensio® Structural Heart 10.2, 3mensio Medical Imaging, B.V., The Netherlands). Direct planimetry of the LVOT area and the diameters were measured in the enface and zoomed view as vertical (Minimum = Dmin) and horizontal (Maximum = Dmax) on the mid-late systole (30–40% systolic phases) (Figure 1). The diameters were used to calculate the LVOT eccentricity index. The eccentricity index was calculated to define the shape of the LVOT (circular or elliptical) using the formula $[1 - (\text{Dmin}/\text{Dmax})] \times 100$. LVOT is considered circular when the eccentricity index was <10% [12].

2.4. Corrected Continuity and Flow Equations

The EOA and SV were calculated using the conventional 2D-TTE-derived parameters; post-TAVI EOA $_{\text{TTE}}$ = [(LVOT area $_{\text{TTE}} \times$ PWD VTI_{LVOT})/CWD VTI_{AV}]. Post-TAVI SV (SV_{TTE}) was calculated as SV_{TTE} = [(LVOT area $_{\text{TTE}} \times$ PWD VTI $_{\text{LVOT}}$).

On the other hand, the corrected equations indicate the use of the MDCT-derived 3D-LVOT area (Direct planimetry from MPR views without geometric assumptions) to be used in the calculation of AV EOA and SV instead of the TTE-derived LVOT area (based on the assumption of circular LVOT shape). Therefore, the corrected parameters were calculated as follows.

$$\text{Post-TAVI EOA}_{\text{MSCT}} = [(\text{LVOT area}_{\text{MDCT}} \times \text{PWD VTI}_{\text{LVOT}})/\text{CWD VTI}_{\text{AV}}]$$

Post-TAVI SV_{MSCT} = [(LVOT area $_{\text{MDCT}} \times$ PWD VTI $_{\text{LVOT}}$). In addition, EOA and SV were indexed to patients' BSA to calculate the indexed corrected parameters.

We compared the PPM rate between the 2D-TTE and the 3D-Corrected-MDCT CE-derived EOAi values.

2.5. Statistical Analysis

Results are presented as mean ± standard deviation (SD) or median with interquartile range (IQR), according to their distribution pattern. We used the Shapiro–Wilks test as well as QQ plot to assess the normality of continuous variables. Categorical data were presented as percentages and fractions of occurrence. Correlation and agreement between the LVOT area and LVOT area dependent parameters, obtained by different methods (2D TTE and MDCT), were determined using Pearson correlation, Spearman rank correlation and Bland–Altman analysis, respectively. Correlation and agreement between mean trans prosthetic PG, with the EOA and EOAi, calculated from TTE and MDCT.

Intra-observer and inter-observer (two independent blinded observers) reproducibility of LVOT area measured by TTE and MSCT was performed in a random set of 20 patients and evaluated using the intraclass correlation coefficient for absolute agreement. Good agreement was defined as >0.80. Mean transprosthetic PG was scatter-plotted for each imaging-technique-derived EOA and EOAi and fitted curves for data pairs were constructed.

The Bayesian mixed-effect model was used to account the cluster effects of measurements, while parameters obtained from two methods are nested within patients.

Bayesian mixed-effect models with gaussian and asymptotic Laplace priors based on the distribution pattern of the dependent variables were used to compare the quantitative parameters between the two groups. While gaussian and asymptotic Laplace were used for normal and skewed distribution, respectively. Furthermore, Bayesian mixed-effect models with Bernoulli (binary) and cumulative priors (ordinal) were used to compare the PPM rate between the two methods. We also used the mixed-effect Bayesian regression model to compare changes in the hemodynamic performance of ACURATE *neo2* among small (23 mm), intermediate (25 mm) and large (27 mm) sizes after implantation.

The convergence of the Bayesian models was examined using R-hat, LOO, and posterior predictive plots. The R-hat < 1.1 indicates a suitable model of convergence. All statistical analyses were conducted using the ggplot2 and rstan packages in the R 4.1.1 environment.

The posterior Beta or Odds ratio (OR) was used to report the associations between variables of interest. The 95% credible interval (Crl) was used to examine the differences

between the two groups, Crl includes zero value for continuous models and one for categorical models indicating non-significant associations.

3. Results

A total of 554 patients with severe AS were treated with TAVI using ACURATE *neo2* between September 2020 and April 2021 and included in the Early neo2 Registry. We excluded patients with MDCT without mid–late systolic phases, patients who required valve in valve bailout therapy with a device other than ACURATE *neo2*, and patients without either post-TAVI 2D TTE study or pre-TAVI MDCT available in the Core Lab for the independent analysis. In total, 258 patients comprised the final cohort of this study. Mean age was 81.6 ± 6.1 years, 65% women with a median of the European System for Cardiac Operative Risk Evaluation (EuroSCORE) II of 3.34% [2.15, 3.5]. The baseline characteristics of the study population are shown in Table 1. The median duration between the pre-TAVI MDCT scan and the TAVI procedure was 13 days [2, 46]. The study cohort included eight patients (3.1%) with type I bicuspid AV, and six patients with TAVI in malfunctioning surgical AV prosthesis (TAVI in SAVR). Pre-TAVI MDCT scans analysis revealed a mean of the native annulus area of 430.2 ± 62.9 mm^2, LVOT minimum and maximum diameters of 19.03 ± 2.55 mm, and 26.92 ± 2.43 mm, respectively with a measured LVOT area of 405.22 ± 81.32 mm^2, and LVOT eccentricity index 29.21 ± 7.4% indicating that LVOT area was oval (Elliptical) in 257 (99.5%) cases (Table 1). All patients were treated via transfemoral vascular access, balloon pre-dilatation was performed in 81.8%, while post-dilatation was performed in 41.1% (Table 2).

Table 1. Patients' baseline characteristics, pre-procedural Echocardiography and MDCT scan.

Characteristic	*n* = 258
Age	81.6 (6.1)
Women	168 (65%)
Body surface area, m^2	1.8 [1.7–2.0]
Body mass index, kg/m^2	26 [23.7–29.3]
Body mass index < 30 kg/m^2	204 (79%)
Body mass index ≥ 30 kg/m^2	54 (21%)
Euroscore II, %	3.34 [2.15–3.5]
Hypertension	212 (82.2%)
Diabetes mellitus Type I	32 (12.4%)
Diabetes mellitus Type II	49 (19%)
Baseline creatinine, mg/dL	1.0 [0.8–1.3]
Prior Atrial fibrillation	102 (39.5%)
Chronic lung obstructive disease	39 (15.1%)
Prior stroke or TIA	33 (12.8%)
Peripheral arterial disease	30 (11.6)
Prior permanent pacemaker implantation	26 (10.1%)
Previous cardiac surgery	30 (11.6%)
Previous CABG	15 (5.8%)
Previous PCI	51 (19.8)
New York Heart Association (NYHA) class	
Class II	86 (33.3%)
Class III	141 (54.7%)
Class IV	25 (9.7%)
Valve-in-Valve procedure (TAVI-in-SAVR)	6 (2.3%)
Preprocedural 2D-TTE characteristics	
LV Ejection fraction, %	60 [55–65]
Aortic valve maximum velocity, m/s	4.29 (0.56)
Mean pressure gradient, mmHg	43.6 [35–52]
Aortic valve effective orifice area, cm^2	0.7 [0.6–0.8]
Moderate-severe aortic regurgitation	28 (10.9%)
Moderate-severe mitral regurgitation	38 (14.8%)
Moderate-severe tricuspid regurgitation	21 (8.2%)

Table 1. *Cont.*

Characteristic	*n* = 258
Pre-procedural MDCT characteristics	
Bicuspid Aortic Valve (Type I)	8 (3.1%)
Native aortic annulus area, mm^2	430.2 (62.9)
MDCT-derived LVOT measurements	
Minimum diameter, mm	19.03 (2.55)
Maximum diameter, mm	26.92 (2.43)
LVOT area, mm^2	405.22 (81.32)

Values are either Median [IQR], Mean (±SD), and *n* (%).

Table 2. Procedural characteristics and In-hospital outcomes.

	***n* (%)**
Vascular access	
Transfemoral	258 (100%)
Balloon pre-dilatation	211 (81.8%)
ACURATEneo2 size	
Small {23 mm}	59 (22.9%)
Medium {25 mm}	101 (39.1%)
Large {27 mm}	98 (38%)
Balloon post-dilatation	106 (41.1%)
Valve embolization	1 (0.4%)
Need for second valve implantation	1 (0.4%)
Annular injury (rupture)	0
Cardiac tamponade	0
Procedural death	0
Coronary obstruction	0
New postoperative permanent pacemaker	18 (7%)
Major vascular complications	4 (1.6%)
Major bleeding	4 (1.6%)
Life-threatening bleeding	3 (1.2%)
In-hospital stroke	7 (2.7%)
Conversion to surgery	0
New dialysis	0
All-cause mortality	0

Values are presented as *n* (%).

3.1. Hemodynamic Outcomes (Conventional 2D TTE and MSCT-Corrected Parameters)

Post-procedural 2D-TTE assessment revealed LVEF of 58.9 ± 9.8%, AV maximum velocity 1.98 ± 0.44 m/s, trans-prosthetic mean pressure gradient 7.22 ± 3.11 mmHg and dimensionless velocity index (DVI 0.64 ± 0.13. Post-TAVI residual AR assessment revealed 59.7% of patients with none/trace AR, 36.4% had mild AR, 1.9% with moderate AR and none had severe AR (Table 3).

The mean LVOT diameter on 2D-TTE was 21.03 ± 1.9 mm and shows a significant difference between the LVOT dimensions obtained from the MDCT scan, Dmin 19.03 ± 2.55 mm (95% CrI of differences: 1.7, 2.31) and Dmax 26.92 ± 2.43 mm (95% CrI of differences: −6.2, −5.58).

The mean LVOT area obtained from TTE and MDCT were 350.4 ± 62.04 mm^2 and 405.22 ± 81.32 mm^2, respectively (95% CrI of differences: −55.15, −36.09), which resulted in a smaller EOA and lower SV (2.25 ± 0.59 vs. 2.58 ± 0.63 cm^2) and (73.88 ± 21.41 vs. 84.47 ± 22.66 mL), (Beta = −0.642 (95%CrI of differences: −0.85, −0.43), (Beta = −7.29 (95% CrI: −14.45, −0.14)), respectively and consequently the indexed values (EOAi 1.20 ± 0.32 cm^2/m^2 vs. 1.41 ± 0.34 cm^2/m^2 (95% CrI of differences: −0.207, −0.136), SVi TTE 41 ± 12.6 mL/m^2 vs. 46.14 ± 12 mL/m^2 (95% CrI of differences: −0.207, −0.136) (Table 4).

Table 3. Post-procedural TTE-Doppler assessment.

	TTE (n = 258)
LV ejection fraction, %	58.9 (9.8)
AV maximum velocity, m/s	1.98 (0.44)
AV mean pressure gradient, mmHg	7.22 (3.11)
Dimensionless velocity index	0.64 (0.13)
Systolic pulmonary artery pressure, mmHg	36.8 [29.5–44.1]
Post-TAVI aortic regurgitation	
None/trace	154 (59.7%)
Mild	94 (36.4%)
Moderate	5 (1.9%)
Moderate–severe mitral regurgitation	36 (15.2%)
Moderate–severe tricuspid regurgitation	49 (24.5%)

Values are either Median [IQR], Mean [±SD] and *n* (%).

Table 4. LVOT-dependent hemodynamic parameters (TTE- vs. MDCT-derived LVOT area).

	TTE	MSCT	95% CrI of Difference
LVOT diameter, mm	21.03 (1.9)	Minimum diameter 19.03 (2.55) Maximum diameter 26.92 (2.43)	[1.7, 2.31] [−6.2, −5.58]
LVOT area, mm^2	350.4 (62.04)	405.22 (81.32)	[−55.15, −36.09]
EOA, cm^2	2.25 (0.59)	2.58 (0.63)	[−0.85, −0.43]
EOA index, cm^2/m^2	1.20 (0.32)	1.41 (0.34)	[−0.207, −0.136]
LVOT SV, mL	73.88 (21.41)	84.47 (22.66)	[−14.45, −0.14]
LVOT SV index, mL/m^2	41.0 (12.6)	46.14 (12)	[−0.207, −0.136]
Prosthesis-Patient Mismatch (PPM)			
- All PPM	22 (8.52%)	6 (2.32%)	
- BMI adjusted PPM			
BMI < 30 kg/m^2			
Moderate PPM	15 (7.3%)	5 (2.5%)	
Severe PPM	5 (2.5%)	1 (0.5%)	
BMI ≥ 30 kg/m^2			
Moderate PPM	2 (3.7%)	0	
Severe PPM	0	0	

Values are either Median [IQR], Mean (±SD) and *n* (%); BMI = Body Mass Index.

3.2. Prosthesis-Patient Mismatch (PPM) Incidence and Reclassification

The incidence of all (overall) PPM measured by conventional 2D-TTE (8.52%) was higher than MDCT-corrected formula (2.32%), OR = 8.36 (95% Crl: 2.42, 39.61), (Kappa w = 0.323, 95% confidence interval (CI): 0.13, 0.51). However, the differences remained statistically significant in the adjusted model by sex, age, and BMI variables (OR = 10.33; 95% CrI: 2.5, 67.34). The distributions of PPM frequency within BMI categories are shown in Figure 2.

3.3. Stroke Volume Index Changes in Patients with Low EF%

In 29 patients with EF < 50% (mean of EF was 40.47 ± 6.49%), the SVi changed significantly from 34.1 ± 11.4 mL/m^2 by TTE to 39.3 ± 11 mL/m^2 with MDCT LVOT-corrected calculation (Beta = 5.18; 95% CrI: 2.36, 8).

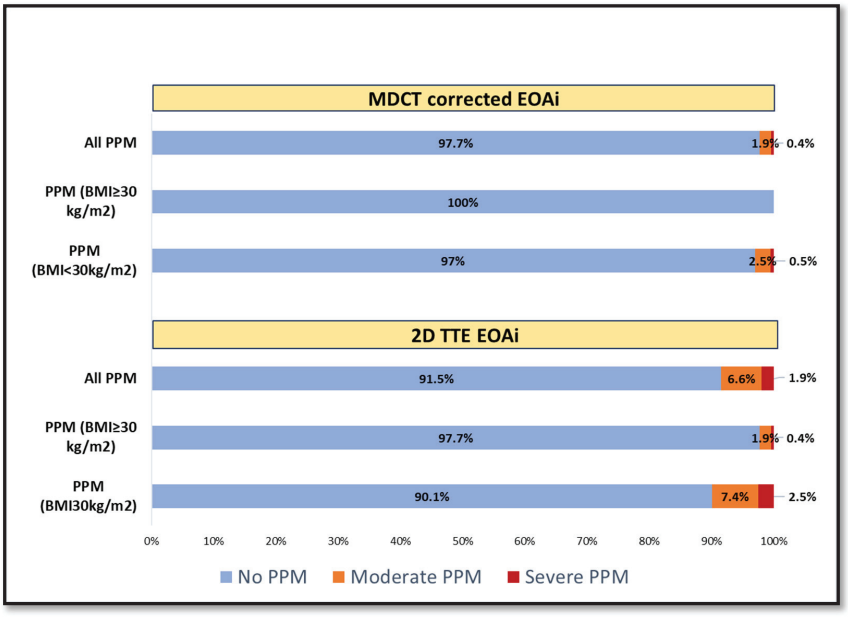

Figure 2. Incidence of PPM (BMI adjusted) based on the EOAi assessed by 2D-TTE and MDCT-corrected method; (All PPM; moderate or severe (2.3% (MDCT-corrected) vs. 8.5% (2D-TTE).

3.4. Inter Valve Size Differences in Hemodynamic Performance and Incidence of PPM

According to the results of the mixed-effects model adjusted for age, sex, BMI, and BSA, to determine the effect of other variables on MDCT and TTE, the detection ability of the interaction effects between methods and independent variables was tested (Table S1).

The EOA*ACURATE *neo2* size interaction was statistically significant; thus, subgroup analysis according to ACURATE *neo2* sizes indicated that mean differences in EOA between TTE and MDCT were obvious for the 23 mm (diff = 0.64, 95% CrI: 0.44, 0.85) compared with the 25 mm (diff = 0.208, 95% CrI: 0.03, 0.35) and 27 mm (diff = 0.26, 95% CrI: 0.11 0.44). The interaction effects between methods and the rest of the independent variables were insignificant. (Figures S4 and S5).

In the simple Bayesian logistic regression model, a higher risk of PPM was observed for ACURATE *neo2* size 23 mm than ACURATE *neo2* size 25 and 27 mm (OR = 3.57; 95% CrI: 1.12, 12.2) with 2D-TTE. With the MDCT, there was no association between the size of ACURATE *neo2* and PPM (Table S1 and Figure S4).

3.5. Intra-Observer and Inter-Observer Reliability

An excellent agreement was observed for the intra-observer and Inter observer reliability regarding LVOT area measured by MDCT (ICC = 0.99 [95% CI; 0.98–0.99]) and (ICC = 0.98 [95% CI; 0.95 to 0.99]), respectively and was good regarding TTE (ICC = 0.87 [95% CI; 0.71, 0.95]) and (ICC = 0.85 [95% CI; 0.63, 0.94]) (Figure S2).

4. Discussion

This is the first study that systematically evaluates the new supra-annular ACURATE *neo2* THV hemodynamic performance using the LVOT area derived from both the conventional 2D-TTE and pre-procedural MDCT scan 3D measurements. Both techniques were used to calculate all LVOT-dependent hemodynamic parameters (EOA, SV in addition to their indexed values) aiming to accurately report hemodynamic parameters outcome early at patients' hospital discharge and to define the baseline hemodynamic performance of

further follow up in comparison to the obtained values, especially for the diagnosis and severity of PPM.

The main findings of this report are as follows; (1) the calculation of LVOT area from 2D-TTE significantly underestimated the area in comparison to the MDCT measured 3D-LVOT area (350 vs. 405 mm^2), and all LVOT-dependent parameters; (2) furthermore, the LVOT was oval in most cases (99.5%) with a mean eccentricity index of 29.2%; (3) recalculation of EOAi resulted in a significant reduction in PPM incidence among the included cohort (8.5% to 2.3%), (4) 3D-MDCT-corrected LVOT are measurements resulted in obtaining more concordance between EOA and other hemodynamic parameters; and (5) finally, the results also show a significant difference between the different sizes with the use of the corrected LVOT assessment in contrast to the conventional TTE assessment.

The fact of measuring a 3D structure using a 2D image usually carries the risk of inaccurate assessment. In LVOT area measurements, our results confirm significant underestimation of the LVOT area in agreement with multiple reports. Liu et al. compared LVOT area measurements using biplane versus single dimensions using TTE, and resulted in the LVOT with the biplane method being larger than the conventional method (420 vs. 373 mm^2) [13]; in addition, Weber et al., have reported that the MDCT-derived LVOT area was larger than 2D-TTE (456.9 vs. 303.7 mm^2) and resulted in larger EOA in patients with severe AS and reclassification of 30% of the included cohort from severe to moderate AS [14].

The concept of using accurately measured LVOT (3D-LVOT) to be included in the CE is a quite old seeking more accurate and reproducible results [15–17], but the application and the use of 3D-LVOT area (3D echocardiography, MDCT, or CMR) area combined with TTE Doppler (CWD and PWD) to obtain AV EOA and SV (corrected parameters) still uncommon practice. However, it could be used especially if discordance in the parameters was noticed either pre- or post-AV replacement or when PPM is suspected [15–17]. Multiple reports confirm the utility of the corrected calculation of EOA using the LVOT area measured from MDCT scan either pre- or post-AV replacement or even for the prediction of EOA, and mainly for the diagnosis of PPM [12,14,18]. The incidence of overall and/or severe PPM after TAVI was reported to be lower than SAVR [19,20], especially with self-expandable, supra-annular devices with larger EOA and lower gradients [21].

The incidence of PPM according to the MDCT-corrected EOAi resulted in a lower frequency of all PPM and BMI-adjusted PPM than 2D-TTE (8.5% vs. 2.3%). (Figure 2 and Table 4) the results agree with those of Fukui et al., who reported larger EOAi (1.57 vs. 1.1 cm^2/m^2) and reclassification of all PPM from 19.5% by TTE to 3.5% with MDCT 3D-LVOT correction for both SEV and BEV [12]. As larger devices are expected to provide larger EOA, a sub-analysis has been performed according to the implanted ACURATE *neo2* size, revealed a significant difference in EOA between the medium, 25 mm, and large, 27 mm, sizes in comparison with the small, 23 mm, devices when the MDCT-corrected LVOT area was used instead of the 2D-TTE LVOT area, which showed a non-significant difference (Table S1, Figure S4).

This study recommends that LVOT area should be directly measured on a 3D imaging modality such as MDCT in all cases, if possible. Correlation of this study findings of misclassified PPM cases might offer an explanation on the lack of clinical correlation of PPM following TAVI in earlier publications. Those cases were most probably misclassified due to underestimation of LVOT area, and consequently EOA.

Study Limitations

Although this is the first study to provide comprehensive hemodynamic reassessment and describes the recalculation of EOA and SV after implantation of the ACURATE *neo2* THV using the MDCT-derived LVOT area, some limitations exist. First, this is a retrospective study with small sample size. Second, long-term clinical outcomes of the PPM reclassification between the two methods are not available. Third, we used the same cutoff values established for the TTE assessment. Therefore, new cut-off values of PPM based on

3D-derived EOA should be derived from long-term outcome studies, and finally, the use of the pre-TAVI MDCT to measure the 3D-LVOT area, but we thought that with the short time interval between the pre-procedural MDCT and the TAVI procedure (13 days) and the post-procedural TTE, no significant changes in the LV mass will occur. Additionally, the low radial force of the ACURATE *neo2* will not significantly affect the shape of the LVOT.

5. Conclusions

LVOT is eccentric in most patients undergoing TAVI, which might lead to erroneous estimation of hemodynamic performance of THV from 2D-TTE using the continuity equation. Using the directly measured LVOT area on a 3D MDCT scan, instead of 2D-TTE, in combination with the TTE Doppler might reduce these limitations, and could result in an accurate and reproducible assessment of continuity-equation-derived parameters. The correction of the LVOT area showed a lower rate of PPM diagnosis dependent on the EOAi, resulting in a better correlation with other hemodynamic parameters, such as mean gradient. Using the MDCT-corrected measurements, the ACURATE *neo2* THV, a self-expandable supra-annular valve, provides a very low rate of PPM, a large EOA associated with a low trans-prosthetic pressure gradient.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm11206103/s1, Figure S1: Bland–Altman plot showing the difference in calculated LVOT area, EOA, SV and EOAi using the 2D TTE- and MDCT-corrected method. Figure S2: MDCT LVOT measurement; Interobserver and Intra-observer reliability with excellent ICCs. Figure S3: Scatter plots of the mean transvalvular gradient and EOA-2D TTE-, EOAi-2D TTE-, MDCT-corrected EOA, MDCT-corrected EOAi. Figure S4: PPM (BMI adjusted) incidence, assessed by the 2D TTE- and MDCT-corrected methods and classified per ACURATEneo2 size. Figure S5: Inter valve size difference according to EOA, EOAi, SV and SVi per valve size; Table S1: Comparison among ACURATEneo2 sizes and their interactions with methods (2D TTE & MDCT); The results of adjusted multiple Bayesian regression model.

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N-Terminal of the Prohormone Brain Natriuretic Peptide Predicts Postoperative Cardiogenic Shock Requiring Extracorporeal Membrane Oxygenation

Piotr Duchnowski

Cardinal Wyszynski National Institute of Cardiology, Alpejska 42, 04-628 Warsaw, Poland; pduchnowski@ikard.pl

Abstract: Aims: Heart valve surgery is associated with a risk of serious postoperative complications including postoperative cardiogenic shock (described as postcardiotomy shock (PCS)). The indication for extracorporeal membrane oxygenation (ECMO) is cardiogenic shock, which is resistant to optimal causal and pharmacological treatment, including the supply of catecholamines and/or an intra-aortic balloon pump (IABP). The aim of this study was to assess the usefulness of the selected preoperative biomarkers in the prediction of postoperative cardiogenic shock requiring ECMO in patients undergoing heart valve surgery. Methods: A prospective study was conducted on a group of consecutive patients with significant valvular heart disease that underwent elective valve surgery. The primary endpoint at the intra-hospital follow-up was postoperative cardiogenic shock requiring ECMO. Univariate analysis, followed by multivariate regression analysis, were performed. Results: The study included 610 patients. The primary endpoint occurred in 15 patients. At multivariate analysis, the preoperative N-terminal of the prohormone brain natriuretic peptide (NT-proBNP) level (OR 1.022; 95% CI 1.011–1.034; $p = 0.001$) remained an independent predictor of the primary endpoint. Conclusions: An elevated NT-proBNP level was associated with a higher risk of postoperative cardiogenic shock requiring the use of ECMO.

Keywords: NT-proBNP; cardiogenic shock; valve surgery; extracorporeal membrane oxygenation (ECMO)

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1. Introduction

Heart valve surgery is associated with a risk of serious postoperative complications including postoperative cardiogenic shock (described as postcardiotomy shock (PCS)) [1–4]. PCS is poorly defined in the literature, but it is broadly understood to mean circulatory failure after cardiac surgery which is resistant to inotropic support and/or an intra-aortic balloon pump (IABP), and which requires mechanical circulatory support (MCS) such extracorporeal membrane oxygenation (ECMO). ECMO may improve a patient's physiological state when in cardiogenic shock by stabilizing hemodynamics and tissue metabolism, allowing the necessary time for regeneration of the heart muscle [5–8]. The available literature has little information on postcardiotomy shock in patients undergoing heart valve surgery. Previous studies have indicated that surgery on cardiac arrest, reduced preoperative left ventricular systolic function, or high-sensitivity troponin T (hs-TnT) measured immediately after surgery are predictors of postoperative cardiogenic shock [9,10]. The N-terminal of the prohormone brain natriuretic peptide (NT-proBNP) is a prohormone secreted into the blood mainly by left ventricular cardiomyocytes, which participates in the maintenance of cardiovascular homeostasis. The active form of the hormone—brain natriuretic peptide (BNP)—causes an increase in glomerular filtration, a decrease in sodium reabsorption in the kidney, the inhibition of aldosterone secretion, and a decrease in the activity of the sympathetic system. The process of NT-proBNP secretion by cardiomyocytes occurs in response to an increase in their voltage in the course of increased pre- and/or after-load. An increase in the concentration of BNP and the prohormone NT-proBNP in

the blood is demonstrated, among other things, by the activation of the compensation mechanism, which already occurs in the period preceding the appearance of symptoms of heart failure [11–14]. The predictive ability of NT-proBNP has been reported in numerous publications on various cardiovascular disorders, including aortic stenosis, heart failure, coronary artery disease, myocardial infarction, congenital heart disease, and postoperative hemodynamic instability [15–21]. However, there is no information regarding NT-proBNP as a predictor of persisting cardiogenic shock despite intensive conservative treatment including the use of catecholamines and/or an IABP. Therefore, in the presented study, we attempted to assess the usefulness of NT-proBNP levels in predicting cardiogenic shock requiring ECMO.

2. Methods

This is a prospective study of consecutive patients with hemodynamically significant valvular heart disease (aortic stenosis, aortic regurgitation, mitral stenosis, or mitral regurgitation) that were approved for heart valve surgery and subsequently underwent elective replacement or repair of the valve/valves, with or without a concomitant coronary artery bypass graft (CABG) at the National Institute of Cardiology, Warsaw, Poland. The exclusion criteria were a lack of consent to participate in the study, patients under 18 years of age, porcelain aorta, active infective endocarditis, and active neoplastic diseases. The day before surgery, a blood sample was collected from each patient. The plasma levels of the NT-proBNP concentrations were measured by electrochemiluminescent immunoassays using Elecsys 2010 (Roche, Mannheim, Germany) and the plasma levels of the cardiac TnT (cTnT) concentrations were measured by the troponin T hs-STAT (Roche, Mannheim, Germany). All procedures were performed through a midline sternotomy incision under general anaesthesia in a normothermia state. All patients were given cold blood cardioplegia at an initial dose of 15–20 mL/kg, followed by booster doses of 5–10 mL/kg every 20 min. The primary endpoint at intra-hospital follow-up was postoperative cardiogenic shock requiring the use of ECMO. Postoperative cardiogenic shock was diagnosed in patients with a systolic blood pressure of below 90 mm Hg and symptoms of organ hypoperfusion (cold viscous skin, altered mental state, oliguria, and increased serum lactate level) that were resistant to inotropic support and/or an IABP. The decision to use the arterio-venous ECMO was made by the team of physicians responsible for the patient who was observed to continue cardiogenic shock despite intensive conservative treatment, including the use of catecholamines and possibly an IABP. Patients were observed until discharge from the hospital or until death. The protocol was approved by the Institutional Ethics Committee of the Institute of Cardiology, Warsaw, Poland (number 1705).

3. Statistical Analysis

Statistical analysis was performed with IBM SPSS software, version 2.0 (SPSS Inc., Chicago, IL, USA). Data are presented as medians with ranges if continuous, or as frequencies if categorical. Binary logistic regression was used to assess the relationships between the variables. The following preoperative covariates were investigated for association with the primary endpoint in univariate analysis: age, BMI, bilirubine, creatinine, hs-TnT, NT-proBNP, haemoglobin level, red cell distribution width, left ventricular ejection fraction (LVEF), New York Heart Association (NYHA) classes, heart rate before surgery, blood pressure before surgery, tricuspid annular plane systolic excursion (TAPSE), right ventricular systolic pressure (RVSP), atrial fibrillation, moreover aortic cross-clamp time, cardiopulmonary bypass time, aortic valve plasty (AVP), aortic valve replacement (AVR), CABG, mitral valve plasty (MVP), mitral valve replacement (MVR), AVR plus MVR and major blending after surgery for the entire patient group, and patient subgroups with and without atrial fibrillation. Significant determinants ($p < 0.05$) identified from the univariate analysis for the entire patient group were subsequently entered into multivariate models. The predictive values of NT-proBNP were assessed by a comparison of the areas under the receiver operator characteristics of the respective curve. On the basis of the Youden

index, a cut-off point was determined that met with the criterion of maximum sensitivity and specificity for mortality prediction. Spearman's rank correlation test was performed to examine possible associations between the variables describing the severity of heart failure and myocardial damage, that is, the test for associations between the values of NT-proBNP and NYHA classes, LVEF, TAPSE, and hs-TnT.

4. Results

The present study included 610 patients undergoing heart valve surgery, with or without concomitant procedures on coronary arteries. The mean age in the studied population was 63 (±12). Forty-nine (8%) patients had a significantly impaired LVEF (LVEF ≤ 35%) before cardiac surgery. The mean preoperative NT-proBNP level was 2003 pg/mL (standard deviation (SD) ± 1532). Table 1 presents the characteristics of the entire study group. Postoperative cardiogenic shock requiring ECMO occurred in 15 patients. In eight cases, an IABP was used (these were patients with haemodynamic instability in the immediate postoperative period that did not respond to increased doses of catecholamines before leaving the operating table). Due to the lack of stabilization of hemodynamic parameters, the IABP was replaced with ECMO in five cases. The average ECMO implantation time after the surgery was completed was 18 h. In each case, the indication for ECMO implantation was increasing hemodynamic instability accompanied by an increase in tissue hypoxia markers. The statistically significant predictors of the primary endpoint at univariate analysis are presented in Table 2 (univariate analysis showed a trend towards statistical significance of the cardiopulmonary bypass time parameter for the primary endpoint, $p = 0.07$). At multivariate analysis, only NT-proBNP (OR 1.022; 95% CI 1.011–1.034; $p = 0.001$) remained an independent predictor of the primary endpoint. The area under the receiver operator characteristic curve for the primary endpoint for NT-proBNP was 0.764 (95% CI 0.728–0.797) (sensitivity: 67%; specificity: 79%) (Figure 1). The mean preoperative value of NT-proBNP in patients with postoperative cardiogenic shock requiring ECMO was 7053 pg/mL (±3532) and was significantly higher compared to patients with no postoperative cardiogenic shock 1875 pg/mL (±1430) ($p < 0.01$). A significant correlation was found between the level of preoperative NT-proBNP and NYHA classes (r = 0.33, $p < 0.001$), pre-operative LVEF (r = −0.35; $p < 0.001$), and the level of hs-TnT (r = 0.4; $p < 0.001$). Of the 15 patients who received ECMO for cardiogenic shock, 6 were fatal as a result gradually increasing multiple organ dysfunction syndrome. The mean NT-proBNP value in the group of patients requiring ECMO who died was 10,274 pg/mL (±7628) and was significantly higher compared to the patients requiring ECMO support who survived (5056 pg/mL (±3102)) ($p < 0.05$). The total 30-day mortality was 3.7% versus 3.5% (expected mortality was calculated using EuroSCORE II (www.euroscore.org, 30 April 2022).

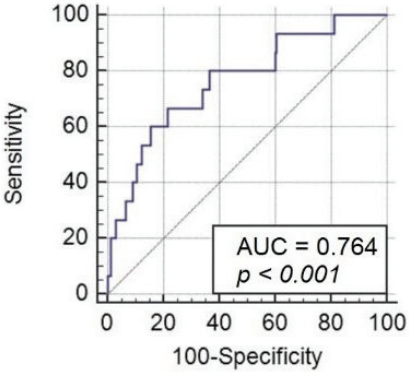

Figure 1. Area under the receiver operating characteristic (AUC) ROC curve of NT-proBNP for cardiogenic shock requiring extracorporeal membrane oxygenation following valve surgery.

Table 1. Baseline characteristics of the study population.

Preoperative Characteristics of Patients (*n* = 610)	Values All Patients	Values Patients with ECMO (*n* = 15)	Values Patients without ECMO (*n* = 595)	*p*-Value
Age, years *	63 ± 12	63 ± 11	65 ± 12	Ns
Male: men, *n* (%)	351 (57%)	8 (53%)	343 (57%)	Ns
Body mass index, kg/m^2 *	28 ± 8	26 ± 6	27 ± 8	Ns
EuroSCORE II, % *	3.5 ± 3.1	3.9 ± 3.5	3.5 ± 3.0	0.04
NYHA, (classes) *	2.5 ± 0.5	3 ± 0.5	2.5 ± 0.6	0.03
LV ejection fraction, % *	57 ± 12	55 ± 12	60 ± 12	0.04
TAPSE, mm *	22 ± 8	21 ± 7	22 ± 7	Ns
RVSP, mmHg *	44 ± 17	48 ± 17	40 ± 16	0.04
Atrial fibrillation, *n* (%)	266 (43%)	6 (40%)	260 (43%)	Ns
Diabetes mellitus, *n* (%)	113 (18%)	3 (20%)	110 (18%)	Ns
NT-proBNP, pg/mL *	2003 ± 1532	7053 ± 3532	1875 ± 1430	0.002
Hs-TnT, ng/L *	34 ± 28	91 ± 58	28 ± 15	0.009
Creatinine, mg/dL *	0.9 ± 0.5	1.4 ± 0.7	0.8 ± 5	0.02
Hemoglobin, g/dL *	13.6 ± 1.5	13.3 ± 1.3	13.8 ± 1.4	0.04
Red cell distribution width, % *	14.2 ± 1.7	15.1 ± 1.7	13.8 ± 1.6	0.009
Intraoperavite and postoperative characteristics of patients:				
AVR, *n* (%)	313 (51%)	7 (46%)	306 (51%)	Ns
AVP, *n* (%)	17 (3%)	Ns	17 (3%)	Ns
MVR, *n* (%)	112 (18%)	3 (20%)	109 (18%)	Ns
MVR + AVR, *n* (%)	53 (9%)	2 (13%)	51 (8%)	0.04
MVP, *n* (%)	115 (19%)	3 (20%)	112 (18%)	Ns
Additional procedureCABG, *n* (%)	90 (14%)	2 (13%)	88 (15%)	Ns
Aortic cross-clamp time, min *	101 ± 32	122 ± 39	98 ± 30	0.01
Cardiopulmonary bypass time, min *	125 ± 55	143 ± 61	118 ± 43	0.03
Postoperative major blending, *n* (%)	47 (8%)	4 (26%)	43 (7%)	0.009

Values are represented by the mean * and a measure of the variation of the internal standard deviation. Abbreviations: AVP: aortic valve plasty, AVR: aortic valve replacement, CABG: coronary artery bypass graft, MVP: mitral valve plasty, MVR: mitral valve replacement, Hs-TnT: high-sensitivity Troponin T, NT-proBNP: n-terminal of the prohormone brain natriuretic peptide, LV: left ventricle, NYHA: New York Heart Association, RVSP: right ventricular systolic pressure, TAPSE: tricuspid annular plane systolic excursion.

Table 2. Univariate analysis of the predictive factors for the primary endpoint.

Variable	Odds Ratio	95% CI	*p*-Value
Hemoglobin, g/dL	0.763	0.625–0.967	0.02
NT-proBNP, pg/mL	1.020	1.009–1.036	0.001
RDW, %	1.327	1.083–1.626	0.006
Cardiopulmonary bypass time, min *	1.048	0.986–1.114	0.07

Abbreviations: NT-proBNP: n-terminal of the prohormone brain natriuretic peptide, RDW: red cell distribution width, * denotes the variable that obtained the value closest to achieving statistical significance ($p < 0.05$).

5. Discussion

The use of ECMO should be considered at an early stage of treatment in a haemodynamically unstable patient after heart valve surgery if that patient has low systolic pressure, low cardiac output, and, as a consequence, insufficient tissue perfusion in which clinical stabilization is not achieved despite the use of conservative treatment in combination with the use of catecholamines [6,22]. The administration of oxygenated blood to the arterial system with appropriate kinetic energy, generated by the ECMO pump, ensures the adequate perfusion of peripheral tissues and relieves the heart muscle by promoting its regeneration [23–25]. Therefore, knowledge of the predictors of postoperative cardiogenic shock that do not respond to pharmacological treatment is extremely important because it enables the identification of patients at risk of this complication, thus enabling the early implementation of ECMO, which increases the patient's chances of survival.

The presented study showed that the level of NT-proBNP determined one day prior to heart valve surgery was an independent predictor of postoperative cardiogenic shock requiring extracorporeal membrane oxygenation, although a significant predictive value in the univariate analyses has also been established for the parameters of the red blood cell system, such as haemoglobin and red cell distribution width. NT-proBNP is a prohormone secreted into the blood by cardiomyocytes (mainly the left ventricle). Due to the fact that the active form of BNP is actively involved in maintaining cardiovascular homeostasis, NT-proBNP is currently widely used in the diagnosis and progression of heart failure [26,27].

In the severe valvular heart defects, there is a pressure and/or volume overload of the left ventricle muscle, which leads to an increase in NT-proBNP secretion by cardiomyocytes [11–13]. Prolonged left ventricular wall overload is the cause of the progressive myocardial degenerative process involving gradual cardiomyocyte necrosis and fibrosis [28,29]. Very high NT-proBNP values present in the blood serum of patients with hemodynamically significant valvular heart disease may indicate the decompensation of an overloaded left ventricular muscle, which can be confirmed by a significant correlation between the NT-proBNP level and NYHA classes, the pre-operative hs-TnT level, and the LVEF demonstrated in this study.

The results of the present study indicated that patients with high preoperative NT-proBNP values undergoing heart valve surgery may be exposed to postoperative severe cardiogenic shock that is resistant to conservative treatment, which will require the use of advanced mechanical circulatory support techniques. The trend toward statistical significance of the cardiopulmonary bypass time parameter for the primary endpoint demonstrated in this study may also indicate that a decompensated myocardium is particularly vulnerable to the adverse effects of the non-physiological conditions prevailing during extracorporeal circulation. Moreover, the results of this study may also suggest that an earlier qualification of a patient for the surgical treatment of heart valve disease with less-advanced myocardial injury may be associated with an improved prognosis.

6. Conclusions

To the best of our knowledge, this is the first report showing the prognostic significance of NT-proBNP measured one day before heart valve surgery in the prediction of cardiogenic shock requiring the use of ECMO in the postoperative period. The results of our research may be helpful in improving the qualifications and perioperative care of patients undergoing heart valve surgery. This was a single-center study that included a limited number of participating patients. In future studies, enlarging the group may allow for confirmation of the results obtained.

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Review

Sex-Related Differences in the Pathophysiology, Cardiac Imaging, and Clinical Outcomes of Aortic Stenosis: A Narrative Review

Abdellaziz Dahou [1], Vikky Awasthi [2], Meriem Bkhache [2], Merouane Djellal [2], Xiaofeng Yang [2], Hong Wang [2] and Rihab Bouchareb [2,3,*]

[1] Cardiology Division, Department of Medicine, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114, USA; adahou1@mgh.harvard.edu
[2] Lewis Katz School of Medicine at Temple University, 3500 North Broad Street, Philadelphia, PA 19140, USA
[3] Center for Metabolic Disease Research (CMDR), Department of Cardiovascular Sciences, Lewis Katz School of Medicine at Temple University, 3500 North Broad Street, Philadelphia, PA 19140, USA
* Correspondence: rihab.bouchareb@temple.edu

Abstract: Aortic stenosis (AS) is the most common valvular heart disease in developed countries, and its prevalence is higher in older patients. Clinical studies have shown gender disparity in the pathogenesis and the progression of aortic stenosis. This disparity has led to several overwhelming questions regarding its impact on the clinical outcomes and treatment of the disease and the requirement of personalized sex-specific approaches for its management. Indeed, aortic stenosis differs in the pathophysiological response to pressure overload created by the stenosis in women compared to men, which would translate into differences in cardiac remodeling and clinical outcomes. Several studies have focused on understanding the differences regarding disease progression according to biological gender and have found that sex hormones play a crucial role. Sex hormones affect many metabolic processes, thus activating crucial cell signaling and energy metabolism through mitochondrial activity. Yet, there is still a significant gap in knowledge on how biological sex influences the pathophysiology of AS. In this review, we have discussed studies that point to the role of sex-related physiological differences in the molecular pathways and the clinical presentation of the disease and outcome in women and men. We used the format of narrative review to review and summarize the body of literature without being systematic but with taking great care of considering the most impactful data available to date on the topic, especially randomized trials, metanalyses, and prospective studies and registries when available, as well as experimental studies with rigorous methodological approaches regarding the basic mechanisms and pathophysiology of the disease in women compared to men. The opinion of the authors on a particular issue or finding was expressed when appropriate for clarification.

Keywords: aortic valve; sex; calcification; echocardiography

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1. Introduction

Aortic stenosis (AS) is the most prevalent valvular heart disease in developed countries, and the only available treatment currently is surgical or transcatheter aortic valve replacement [1,2]. The initial phase of the disease involves the stiffening of aortic valve leaflets, known as the sclerotic phase, caused by excessive deposition of extracellular matrix (ECM) [3], which will then eventually evolve into stenosis, characterized by inflammation, endothelial dysfunction, lipid deposition, and the accumulation of calcium deposits that distort the valve's structure, accompanied by the development of neovascularization [4,5]. This process initially manifests with subtle or no clinical symptoms [6]. The transition from sclerosis to stenosis occurs in about 10–15% of individuals within a period of 2 to 5 years, according to some data [7], but may vary from one patient to another with potential

differences between men and women. Patients with more advanced valve obstruction (moderate vs. mild stenosis) typically undergo a more rapid progression toward severe stenosis, requiring valve replacement [8,9].

Increasing evidence is showing differences in the mechanisms of valvular remodeling and differences in the severity of calcification and fibrosis between men and women [10] (Figure 1), which, in the end, translates into differences in clinical presentation and outcome [11,12]. This highlights the necessity for dedicated sex-specific investigations to understand the underlying mechanisms behind this dimorphism better and enhance treatment effectiveness and outcomes.

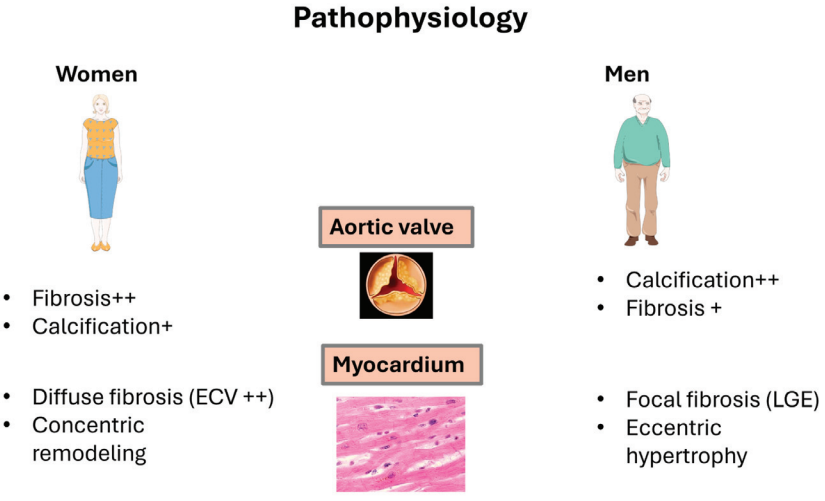

Figure 1. The nature of aortic stenosis differs between men and women. In women, it is marked by increased fibrosis and decreased calcification, while in men, the valve calcification is more severe and more dominant than fibrosis. Furthermore, in women, the progression of aortic stenosis is marked by concentric remodeling of LV with diffuse fibrosis, represented by increased fraction of extracellular volume (ECV), while in men, it presents more eccentric hypertrophy and focal fibrosis, represented by the presence of late gadolinium enhancement (LGE).

2. Biological Sex Hormones and Aortic Valve Cell Calcification

2.1. The Link between Sex Hormones and AS

Aortic valve stenosis has been linked to gender differences, as observed in various epidemiological studies. The prevalence of aortic stenosis is higher in men as compared to women [13]. The disparities in disease outcomes between genders are thought to stem from varying levels of sex steroid hormones, particularly estrogen and testosterone. This potential link between sex hormone signaling and the development and progression of aortic stenosis was evident from studies in mice, which demonstrated the upregulation of genes associated with aortic valve mineralization in males compared to females [14]. In humans, a cohort study conducted in middle-aged and older Finnish men found a positive association between elevated serum testosterone levels and the risk of developing (AS) [15]. Eildermann et al. investigated androgen receptor (AR) expression in human heart tissue. Their findings showed upregulated AR mRNA levels in patients with aortic stenosis compared to healthy controls [16].

Similarly, the role of estrogen in aortic stenosis development is evident from clinical studies, which have reported a lower incidence of cardiovascular disease (CVD), including aortic stenosis, in premenopausal women compared to age-matched males. This disparity narrows and even reverses after menopause, suggesting a protective role of estrogen during reproductive years [17,18] (Figure 2).

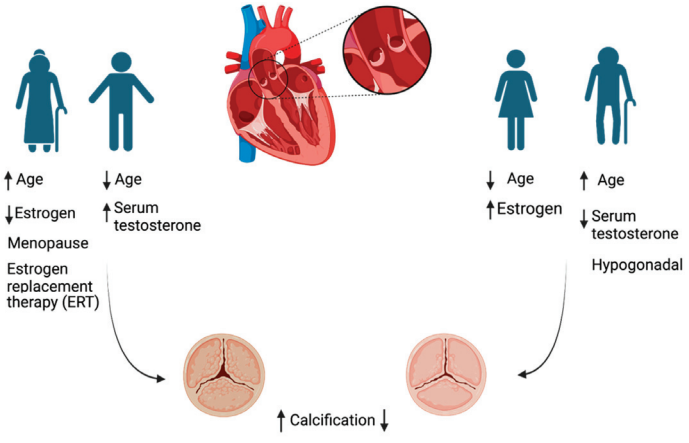

Figure 2. Aortic stenosis in men is characterized by more calcification, while in women, it is characterized by more fibrosis. For both men and women, the incidence of AS increases with age. However, sex hormones significantly impact the progression of AS in men and women. Men develop AS at a younger age compared to women, while the incidence of AS decreases in hypogonadal males. In women, estrogen protects from AS, and when its level decreases with an increase in age and after menopause, the incidence of the disease increases. Furthermore, estrogen replacement therapy (ERT) decreases AS in women.

Sex hormones undoubtedly influence cardiovascular health. Studies utilizing gonadectomized animal models continue to demonstrate sex-related dimorphic disease progression [19]. In an exciting study [1], the hormone effect was well demonstrated in the development of AS using castrated and ovariectomized mice supplemented with respective hormones. The highest hemodynamic progression of AS was observed in intact male mice, which significantly decreased following castration. Interestingly, testosterone supplementation in castrated males partially restored hemodynamic progression, suggesting a dose-dependent effect of androgens on AS severity. This observation aligns with the reported four-fold increase in calcification deposits in intact mice compared to castrated mice. Conversely, the study suggests a minimal role for estrogen in the murine aortic valve pathophysiology. While intact female mice (IF) displayed downregulation of genes, Alkaline Phosphatase and Angiotensin II receptor type 1 associated with valve homeostasis compared to ovariectomized females (OF), supplementation with 17β-estradiol (OFE) did not significantly alter this expression pattern [1].

2.2. Molecular Mechanisms and Sex Hormones in AS

Mechanisms underlying aortic stenosis progression under the influence of sex hormones in humans are still elusive. Sex steroids exert their pleiotropic effects through interactions with distinct receptor subtypes. Estrogen predominantly binds to nuclear estrogen receptors (ERs), including ERα and ERβ. Additionally, it can activate the G protein-coupled estrogen receptor (GPER) [20]. Male sex hormones, testosterone, and its metabolite DHT exert their actions via androgen receptors (ARs) [21]. These receptors exhibit both cytoplasmic and membrane localization, mediating diverse genomic and non-genomic effects involving the activation of multiple intracellular signaling cascades and influencing intracellular calcium (Ca^{2+}) concentration, nitric oxide (NO) synthesis, protein kinase C (PKC), and mitogen-activated protein kinase (MAPK) pathway [22]. These pathways have an instrumental role in the development of aortic stenosis. MAPK signaling is involved in AS [23], while PKC activation inhibits endothelial NO synthase [23], and NO activates NOTCH signaling through S-nitrosylation of USP9X24, preventing AS.

A study showed that culturing valve interstitial cells (VICs) in either a pro-inflammatory or quiescent state did not significantly alter calcium deposition. However, treatment with testosterone (concentration- and duration-dependent) significantly enhanced calcification in both VICs (~16-fold increase) and valve smooth muscle cells (VSMCs) (~5-fold increase) compared to controls, whereas estrogen treatment had no observable effect. Similarly, proteomic profiling of male aortic stenosis patients revealed an upregulation of proteins associated with the extracellular matrix, predominantly proteins of the ITIH family (ITIH1 and ITIH2), which are ECM stabilizers. Additionally, Fibronectin1 and serpine E2 are elevated. Upregulation of serpineE2 leads to increased fibrosis. In contrast, female patients displayed a marked reduction in STAT3, crucial in cardiac fibrosis and collagen synthesis. Several genes upregulated in both genders include proteoglycan small leucine-rich proteoglycan, collagen type I, III, and V, Cartilage Intermediate Layer Protein (*CILP*), and thrombospondin 4 and 5. Further, there is downregulation of proteins involved in cellular energy metabolism like tricarboxylic acid cycle, transporter for long-chain fatty acids (*SLC27A6*), *GLUT1*, fatty acid β-oxidation, branched-chain amino acid catabolism *PYGB*, and glycogen degradation glycolysis-related proteins *PDK4* and *SPTLC*—crucial in the de novo synthesis of ceramides. The proteostasis genes that are upregulated include related genes like Nedd8-conjugating enzyme, *UBE2M*, and *HSPB7* (isoforms 1 and 2), while the *TRiC* and *TRAP1* genes protecting the heart from hypertrophy [24] are downregulated (Figure 3).

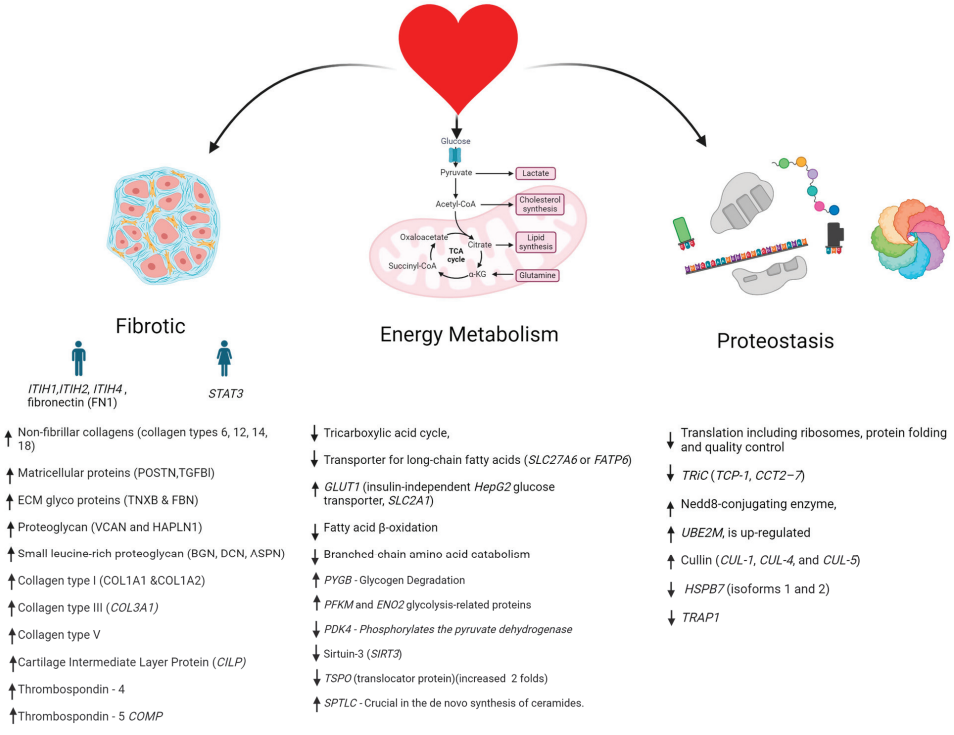

Figure 3. Gender-related protein markers for aortic stenosis. The mechanism of aortic stenosis progression is illustrated with an increase in several molecules related to fibrotic events in the aortic valve, changes in the metabolic status of cells, and proteostasis. During fibrosis, the expression of ITIH1, 2, and 4 and the fibronectin gene increases specifically, while in women, the transcriptional regulator STAT3 increases. Additionally, all genes listed with the upward arrow increase in both biological genders, while the arrows pointing downwards show a decrease in gene expression.

A study by Masjedi et al. [25] showed the presence of sex-related differences in early osteogenic differentiation of the aortic valves at the cellular level. The in vitro study showed an increased proliferation rate in female rat aortic valvular interstitial cells (RAVICS) and porcine aortic valvular interstitial cells (PAVICS) compared to males. In addition, they showed an elevated matrix remodeling: male RAVICS had higher collagen I, Glycosaminoglycan (GAG), and activated matrix metalloproteinase MMP-2 after 15 days of culture in osteogenic media. Moreover, they found an (early osteogenic marker) ALP-positive cells higher in male RAVICS than in females. Male PAVICs have higher calcified nodules after 15 days of culture in osteogenic media. The study also showed an increase in cell proliferation rate in female RAVICS after β-estradiol treatment, while males' proliferation was independent of the treatment amount [25].

2.3. The Role of Testosterone

Testosterone plays a role in calcium homeostasis and can elevate serum calcium levels. Studies in testosterone-deprived male rats have shown that testosterone replacement therapy can attenuate intracellular calcium dyshomeostasis within the heart [26]. Androgens promote calcification [27] and also exert an effect on nitric oxide formation and repress peroxisome proliferator-activated receptor-γ (PPARγ) signaling [28], which influences calcification. In hypogonadal men, low levels of androgens by sustaining Nitric Oxide Synthase (NOS) activity [29,30], reducing nuclear factor κB ligand (RANKL) signaling [31], and reducing transforming growth factor beta (TGFβ) signaling might reduce calcification [32].

It is established that TGFβ plays a pivotal role in AS. Elevated levels of TGF-β1 are observed in patients with AS, and studies in mice demonstrated a positive correlation between plasma TGF-β1 levels and disease severity [33]. Furthermore, depletion of TGF-β1 in platelets has been shown to attenuate AS progression in a mouse model [34]. Investigating the interplay between androgen levels, AR signaling, and TGF-β expression in human aortic stenosis could provide valuable insights into the sex-based disparities observed in this disease, given that AR has been shown to bind to TGF-β promoter, potentially regulating its expression [16,35].

2.4. The Role of Estrogen

Increasing evidence is showing that estrogen is a protective hormone against ectopic mineralization. Furthermore, the post-menopausal estrogen treatments might reduce the risk of cardiovascular calcification if it is administered in the first 5 years of menopause; however, other studies suggest that the initiation of estrogen repletion outside of this period may not confer optimal protection. Indeed, it is dependent on time and estrogen dosing regimens, and further investigations are needed to validate the findings.

Estrogen exerts its biological effects through interactions with ERs. In vitro studies showed that estrogen can reduce L-type Ca^{2+} channel activity, potentially protecting mice from the detrimental effects of elevated intracellular Ca^{2+}, contributing to cardiac hypertrophy via calcineurin (Ser/Thr-phosphatase) activation. Notably, 17β-estradiol administration attenuated pressure-overload hypertrophy development in a rat model of aortic stenosis. This protective effect was associated with inhibited phosphorylation of p38 mitogen-activated protein kinases (MAPKs) [36]. In contrast to androgens, estrogens can suppress a variety of molecular processes known to drive cardiovascular calcification, including repression of receptor-activator of nuclear factor-kappa B ligand (RANKL) [37] and nuclear factor-kappa B (NFκB) signaling and suppression of NADPH oxidase activity in resident cells [38].

2.5. Sex Hormones and Mitochondrial Activity

Sex hormones can regulate mitochondrial activity, thus directly affecting cellular activity [39]. Androgen affects mitochondrial biogenesis by activating the AR/PGC-1α/TFAM pathway [40], autophagy [41], and ATP production [42]. Similarly, estrogen affects mitochondria phospholipid content of membranes, oxidant and antioxidant capacities, oxidative

phosphorylation, and calcium retention capacities [43]. The difference in energy metabolism may significantly shape the outcome of aortic stenosis in men and women.

Finally, although indirect evidence pointing toward a link between sex hormones and AS exists, direct evidence is yet to be established.

3. Sex-Related Differences in Cardiac Imaging in Patients with Aortic Stenosis

Many differences have been highlighted between male and female patients with aortic stenosis (AS). These include anatomical and pathophysiological aspects that involve both the valve leaflets and the ventricular remodeling in response to pressure overload, which, in turn, will translate into differences in imaging findings [44–46].

3.1. Aortic Valve (AV)

Women generally have a smaller AV anatomy, with a smaller aortic annulus and a smaller aortic root. Studies of explanted aortic valves from patients undergoing surgery for AS have shown that men have heavier and more calcified valves than women [47,48]. This difference persisted after adjustment for anatomical factors like the left ventricular outflow tract (LVOT) size and body surface area, regardless of the AV's morphology (tricuspid vs. bicuspid) [48]. Recent studies using multidetector computed tomography (MDCT) to assess AV calcification also showed that, for the same degree of AS severity, men have more severe AV calcification than women, even after adjustment for the size of the aortic annulus [49]. These findings suggest that a lower calcium scoring should be considered in women to diagnose severe AS [49]. This intriguing difference in calcification patterns between men and women has led to further research studies that suggest that women may have more fibrotic remodeling of AV leaflets than men despite having the same degree of AS severity [47]. This difference may be in part related to the hormonal influence highlighted above.

3.2. Left Ventricular (LV) Remodeling

Women are more likely to present with concentric remodeling and a small LV cavity, lower stroke volume despite preserved left ventricular ejection fraction (LVEF), and a more severe diastolic dysfunction due to reduced LV compliance. On the other side of the spectrum, men usually present with eccentric remodeling with less severe diastolic dysfunction [44,46]. According to cardiac magnetic resonance (CMR) studies, women also tended to have higher LVEF than men [50]. CMR studies also showed that women often exhibit lower LV mass compared to men, while data from echocardiographic studies are conflicting [46,51,52].

At the ultrastructural level, studies with advanced imaging techniques like CMR with T1 mapping and data from pathology showed a critical difference between men and women regarding LV remodeling and adaptation to the pressure overload resulting from the AS. Accordingly, women generally have an increased fraction of extracellular volume (ECV), a measure of diffuse myocardial fibrosis. At the same time, men are more likely to present with focal myocardial fibrosis, evidenced by the presence of late gadolinium enhancement (LGE) [50,51]. Diffuse fibrosis (e.g., ECV) is potentially reversible after aortic valve replacement, whereas focal myocardial fibrosis (e.g., LGE) is non-reversible [50,51,53]. The pattern of fibrosis in men with AS appears to be mainly driven by the severity of the AS and the extent of LV hypertrophy. In contrast, it seems to be multifactorial in women [33,45,54–57].

3.3. Clinical Presentation

Women are more likely to present with atypical symptoms such as dizziness, fatigue and dyspnea [58]. This can be due to the differences in pathophysiological adaptations in response to pressure overload in men and women. Women present more frequently with concentric remodeling/hypertrophy and the resulting diastolic dysfunction, while men present more with eccentric remodeling. Associated comorbidities may also play a role in

the clinical presentation. Indeed, women are more likely to present with dysfunction of the coronary microcirculation with atypical presentation while men have a higher prevalence of coronary artery disease (stenosis) which presents as angina during exercise [58,59]. Furthermore, paradoxical low flow, low gradient AS, which presents with a low stroke volume despite a preserved LVEF and a more advanced stage of LV diastolic dysfunction, is more frequent in women and could play a role in the atypical presentation and additional challenges in the diagnosis and grading of AS severity [45].

4. Challenges in Imaging for Diagnosis of AS Severity in Men and Women

4.1. Echocardiography

Doppler echocardiography is the primary modality for the diagnosis and the assessment of AS severity [60]. However, the accuracy of this modality is limited in circumstances that do not allow appropriate visualization of the AV or the accurate measurement of transvalvular gradients, especially when the Doppler beam is not aligned parallel to the direction of the high-velocity jet [60]. In such circumstances, AS severity may be underestimated, which may cause a delay in diagnosis and late referral for treatment. Furthermore, assessment of AS severity may be very challenging in cases with discordance between aortic valve area (AVA) (being severe, i.e., <1 cm^2) and gradient (being non-severe; i.e., <40 mmHg) as in patients with paradoxical low-flow low-gradient (LFLG) AS, which is more frequent in women [60,61]. This aspect becomes even more problematic when the leaflets are less calcified despite severe AS, which is more likely to be seen in women [47,48]. This may lead to late diagnosis and late referral for AV replacement in women, especially if the symptoms are atypical (Figure 4).

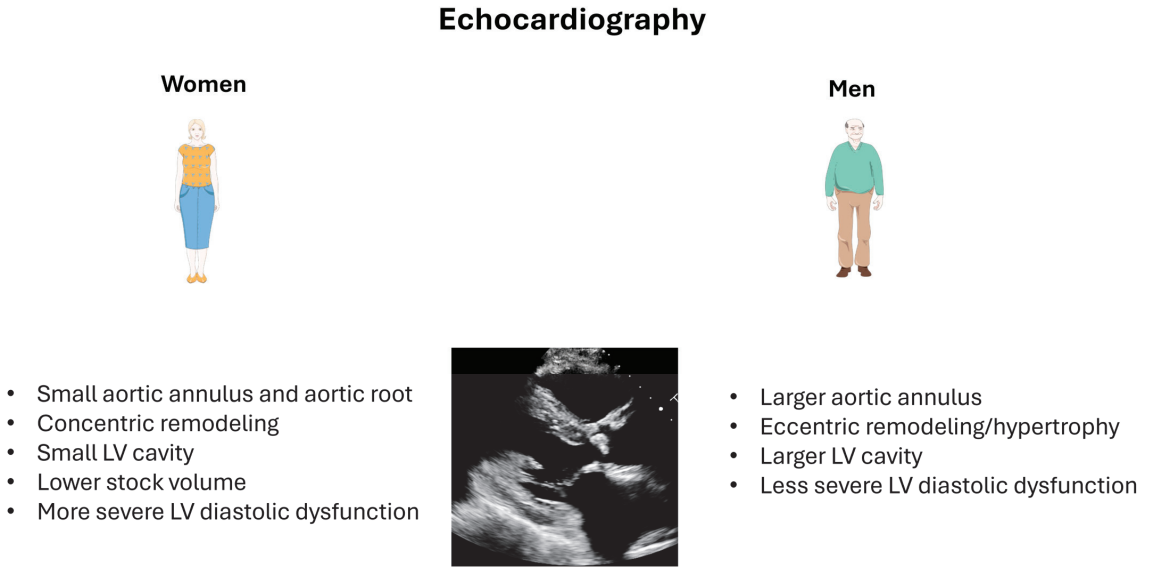

Figure 4. Echocardiographic findings in men and women with aortic stenosis.

4.2. Cardiac Computed Tomography (CT)

Cardiac CT scans remain helpful for assessing AV calcification in patients with AS when assessment of AS severity by Doppler echocardiography is inconclusive [60]. As highlighted above, studies have shown that women exhibit a less severe calcification than men for the same degree of AS severity, and this difference persists even after AV size is adjusted by the size of the aortic annulus and after adjustment for BSA [47–49]. Therefore, current guidelines recommend using different thresholds of calcium scoring in

men and women to diagnose severe AS. Accordingly, a calcium score threshold of ~1300 AU is considered sufficient to diagnose severe AS in women, while a score of ~2000 AU is needed to diagnose severe AS in men (Figure 5) [49,60]. However, due to the critical pathophysiological differences of AV remodeling highlighted above, some cases can present with severe AS despite a calcium score lower than the recommended threshold, especially in women, where the correlation between calcification and AS severity and the weight of the explanted valves—a surrogate of AS severity—was less good in women compared to men [47]. Therefore, one should be careful not to rule out severe AS if the valve leaflets exhibit severe thickening with a severely reduced opening (AVA < 1cm^2) despite a low mean transvalvular gradient (MG < 40 mmHg). This is also true in cases of AS due to or associated with amyloidosis, where AS can be severe despite less severe calcifications due to a more fibrotic remodeling of the valve and the direct infiltration by the amyloidosis process [62]. In these challenging circumstances, further studies, including stress echocardiography when indicated and possible (exercise echo with preserved ejection fraction (EF) and dobutamine stress echocardiogram (DSE) with reduced EF) or, rarely, invasive assessment, are usually needed to confirm AS severity [60].

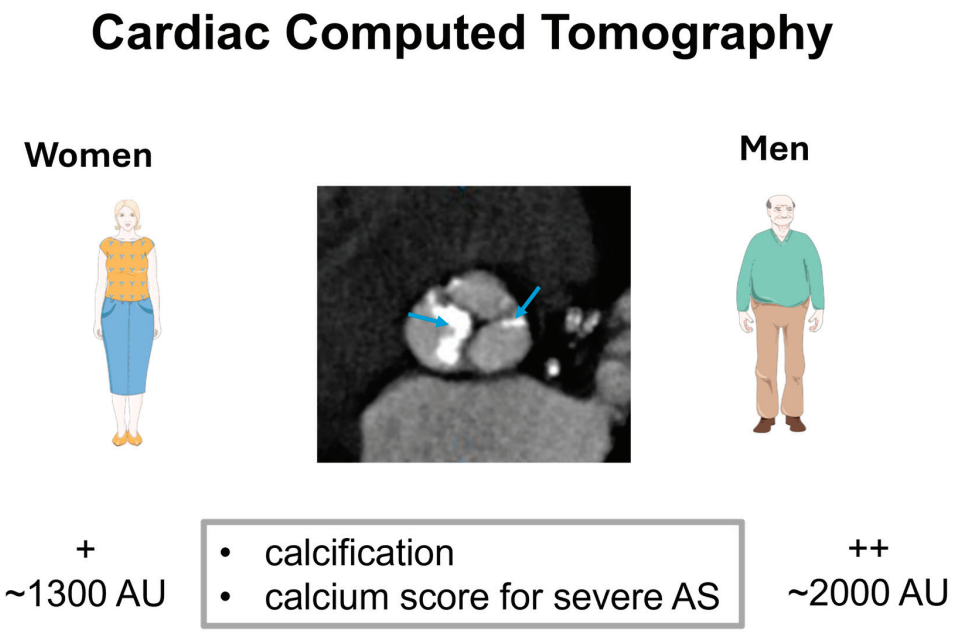

Figure 5. Cardiac computed tomography shows a calcified aortic valve, with a different threshold in men and women for the diagnosis of severe AS. The blue arrows represent the calcification of the aortic valve.

5. Clinical Outcome after Aortic Valve Replacement

The prognosis of AS without treatment is poor [63,64]. The only available treatment is aortic valve replacement (AVR), which can be performed either through surgery (SAVR) or transcatheter aortic valve replacement (TAVR) [65–67]. Recent studies have shown that the 5-year survival after diagnosis of AS was worse in women than in men, and the difference was attributed to a more conservative management of AS in women [68]. Accordingly, women appear to be less likely to be referred for AVR than men, with a later referral, older age, and more advanced stage of the disease at presentation [68,69]. This late referral may be due to multiple reasons, such as an atypical clinical presentation, which may lead to a

late diagnosis, older age at presentation, or a more challenging diagnosis of AS severity, as highlighted above.

Selecting the optimal therapy for each patient depends on several factors, including the patient's anatomy and risk profile, valve durability, and patient preferences [70].

5.1. Transcatheter Aortic Valve Replacement (TAVR)

After both TAVR and SAVR, women appear to have less severe myocardial fibrosis, more favorable left ventricular remodeling, and faster regression of LV hypertrophy than men [71,72].

Overall, among patients who underwent TAVR for severe AS, studies showed no significant difference in 30-day mortality rates between men and women [73–75] despite a greater rate of procedural-related complications in women, like bleeding, vascular complications, and conversion to SAVR [73,76–80]. These complications are, at least in part, a direct consequence of the smaller aortic valve annulus and vascular anatomy and higher rates of porcelain aorta in women than men [81,82]. Recent studies also showed a link between the type of transcatheter valve and the rate of complications, with more common complications in women treated with self-expanding valves [83]. This trend was mainly driven by vascular complications [83].

Several studies, including a recent metanalysis of more than forty-seven thousand patients, showed that despite older age and higher risk profile at the time of TAVR and a higher rate of short-term complications like severe bleeding and vascular complications, women had similar or even better survival compared to men [73,84–87]. This is referred to by some scientists as ''the women paradox". It is worth mentioning though that several baseline factors, but not sex, were predictors of mortality [87].

Although data from previous studies suggest that women may have a more significant benefit from TAVR over SAVR during follow-up [58,73,75–78,88,89], these data are not based on sex-based randomization. One explanation for the potential benefit of TAVR in women as compared to men is thought to be related to a lower prevalence of significant paravalvular regurgitation (PVR)—a powerful prognostic marker following TAVR—as a direct result of less severe AV calcification and a smaller AV annulus size [90]. The presence of PVR can be more problematic in the setting of small LV size and restrictive physiology. This pattern is more observed in women, where even a less severe but acute PVR can have detrimental hemodynamic consequences. This benefit of TAVR over SAVR in women could also be explained by a lower rate of prosthesis–patient mismatch (PPM) following TAVR as compared to SAVR [91] with a more favorable LV reverse remodeling in women after TAVR [73]. The RHEIA trial is a recent prospective, randomized trial that was designed to investigate whether TAVR is superior to SAVR in female patients with severe symptomatic AS, regardless of the surgical risk [92]. The preliminary results of this study presented at the ESC meeting in August 2024 suggest that TAVR was superior to SAVR for reducing death, stroke or rehospitalization, and the benefit was mostly driven by a lower rate of rehospitalizations. TAVR was also associated with a shorter hospital stay compared to SAVR. These data suggest that TAVR could be considered the preferred option to treat women with severe aortic stenosis.

There are some conflicting data regarding pacemaker implantation rates following TAVR [93]. However, a large meta-analysis suggests that women have a lower risk of permanent pacemaker implantation after TAVR [94]. This can also explain the greater benefit of TAVR in women.

However, it is important to remember that long-term data regarding the durability of transcatheter valves are still limited, and further studies are warranted.

5.2. SAVR

Being a biological woman is a prognostic surgical risk factor per se, and it is included in risk scores like the STS score [95]. Although women are at higher risk of complications after surgical AVR [95–98], some studies have shown that despite older age and more

comorbidities, women did not have increased postoperative and long-term mortality after SAVR [96]. However, data from other studies suggest that women undergoing SAVR have a worse 30-day mortality risk compared to men [69,99]. This could be explained by the higher rate in women of vascular complications, bleeding, and blood transfusions with their negative consequences [88]. In addition to a higher rate of prosthesis–patient mismatch due to a smaller aortic annulus in women, and the associated comorbidities like renal and heart failure which makes the prognosis less favorable [74]. Indeed, moderate and severe PPMs are more frequent in women after SAVR and were associated with increased in-hospital mortality as compared to those with no mismatch [100]. PPM was also associated with poorer long-term outcomes, including higher all-cause and cardiac death, heart failure, and rehospitalization [101–104], highlighting the need for the implementation of preventive strategies to avoid PPM after SAVR.

6. Conclusions

In the present review, we discussed the potential sex-specific molecular mechanisms and clinical presentation and outcomes in male and female patients with aortic stenosis. Although important differences between men and women with AS have been highlighted, understanding the underlying mechanisms leading to these differences is still limited. Further studies on the pathophysiology and outcomes of AS in male and female patients are warranted to understand these differences better, personalize the disease's management, and improve the outcomes.

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Review

Aortic Valve Stenosis and Cancer: Problems of Management

Gloria Santangelo [1], Silvia Moscardelli [2], Lucia Barbieri [1], Andrea Faggiano [1,3], Stefano Carugo [1,3] and Pompilio Faggiano [4,*]

[1] Department of Cardio-Thoracic-Vascular Area, Foundation IRCCS Cà Granda Ospedale Maggiore Policlinico, 20154 Milan, Italy; gloria.santangelo@policlinico.mi.it (G.S.); lucia.barbieri@policlinico.mi.it (L.B.); andreafaggiano95@gmail.com (A.F.)
[2] Division of Cardiology, Department of Health Sciences, San Paolo Hospital, University of Milan, 20133 Milan, Italy; silvia@moscardelli.it
[3] Department of Clinical Sciences and Community Health, University of Milan, 20133 Milan, Italy
[4] Fondazione Poliambulanza, Cardiothoracic Department Unit, 25100 Brescia, Italy
* Correspondence: cardiologia@pompiliofaggiano.it

Abstract: Aortic valve stenosis and malignancy frequently coexist and share the same risk factors as atherosclerotic disease. Data reporting the prognosis of patients with severe aortic stenosis and cancer are limited. Tailoring the correct and optimal care for cancer patients with severe aortic stenosis is complex. Cancer patients may be further disadvantaged by aortic stenosis if it interferes with their treatment by increasing the risk associated with oncologic surgery and compounding the risks associated with cardiotoxicity and heart failure (HF). Surgical valve replacement, transcatheter valve implantation, balloon valvuloplasty, and medical therapy are possible treatments for aortic valve stenosis, but when malignancy is present, the choice between these options must take into account the stage of cancer and associated treatment, expected outcome, and comorbidities. Physical examination and Doppler echocardiography are critical in the diagnosis and evaluation of aortic stenosis. The current review considers the available data on the association between aortic stenosis and cancer and the therapeutic options.

Keywords: aortic stenosis; cancer; valve replacement; cardio-oncology; transcatheter valve implantation; radiation therapy

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1. Introduction

The coexistence of cancer and calcific aortic valve stenosis (AS) is a common medical scenario, especially in the elderly, due to sharing risk factors (i.e., hypertension, obesity, diabetes, smoking, dyslipidemia), the inflammatory state associated with malignancies, and/or cardiotoxic effects of cancer therapy [1]. As reported in studies listed in Table 1, the prevalence of cancer in patients with severe AS varies between 5.4 and 37.8% [2–4]. Data reporting the prognosis of patients with severe AS and cancer are limited. In a 10-year single-center retrospective study, cancer patients with severe AS (mean aortic valve area 1.0 ± 0.3 cm^2) had a 5 year mortality of 48%; 59% deaths were due to cancer progression, and 31% were due to heart failure (HF) and stroke [5]. Minamino-Muta et al., in a Japanese retrospective study of 3815 patients in a multicenter AS registry, found that outcomes are worse not only in patients with active cancer but also in those with a previous history of malignancy [6]. Mortality was mainly cancer related, with comparable aortic valve-related deaths between cancer and noncancer patients. Despite the increasing prevalence of AS and cancer, death rates have been steadily declining with the introduction of novel therapies [7], but, at present, the optimal strategy for the management of severe AS in patients with an active cancer is unclear. Cancer patients are routinely excluded from clinical trials because of poor long-term prognosis. Active malignancy often hinders the decision to proceed with invasive procedures, such as cardiac surgery. Furthermore, cancer patients have additional risks due to prior exposure to potentially cardiotoxic chemotherapy, prior chest

radiation, immunocompromised state, and increased risk of both bleeding and thromboembolic disease [8]. In patients with cancer, AS may interfere with optimal antineoplastic management (i.e., high-risk oncological surgery or potentially cardiotoxic chemotherapies). Symptomatic AS is occasionally diagnosed in cancer patients undergoing cardiovascular evaluation; likewise, cancer is often recognized during assessments preceding aortic valve interventions. In these complex cases, physicians face a difficult treatment decision.

Table 1. Prevalence of cancer in patients with severe aortic stenosis according to available studies.

Author	Reference	All Population, n	Cancer, n (%)	Most Frequent Type of Tumor n, (%)
Faggiano et al.	[2]	240	64 (26.6%)	na
Mangner et al.	[3]	1821	99 (5.4%)	Prostate, 25 (25%)
Minamino-Muta et al.	[6]	3815	513 (13.4%)	na
Okura et al.	[5]	26,325	111 (0.42%)	Stomach, 13 (14.1%)
Guha et al.	[4]	47,295	27,960 (37.8%)	na

Usually, an echocardiographic evaluation is done before chemotherapy is started; the presence of LV dysfunction before generally represents a risk situation for chemotherapy; on the other hand, LV dysfunction in the presence of severe AS, especially if symptomatic, represents an indication for the treatment of valvular disease soon.

Khrais et al. found [9] that AS can be seen as a prognostic risk factor for adverse outcomes in patients with colorectal cancer due to higher rates of lower gastrointestinal bleeding and resulting iron-deficiency anemia.

Severe symptomatic AS in patients with cancer requires careful assessment in order to select the appropriate therapeutic choices and their timing (i.e., valve treatment first versus cancer treatment first). First of all, cancer localization and therapy, anemia, self reduction of physical activity, etc. may be important confounding factors in the definition of symptomatic vs. asymptomatic AS. If the stenosis is severe, the dosage of Nt-proBNP/BNP can be useful to attribute the genesis of the symptoms to the valve disease. Echocardiography is key to confirming the diagnosis and severity of AS, assessing valve abnormalities, left ventricular (LV) hypertrophy and function, detecting other valve diseases or aortic pathology, and providing prognostic information. If feasible, exercise testing, especially exercise echocardiography, can clarify the nature of symptoms. These echocardiographic findings must be considered together with coronary/vascular diseases and cardiovascular medications. LV systolic dysfunction represents an important prognostic factor and is included in the current operative risk scores [10]. It can be due to long-standing pressure overload, associated aortic regurgitation or mitral valve disease, coronary artery disease, but, also, to cardiotoxicity induced by cancer treatment (especially anthracyclines and targeted therapies such as tyrosine kinase inhibitor, antihuman epidermal growth factor receptor 2, antivascular endothelial growth factor, or proteasome inhibitors) [11]. Ezaz and colleagues [12] developed a risk-factor-scoring tool for patients on trastuzumab to help identify those at highest risk of developing HF or cardiomyopathy. A seven-factor risk (age, adjuvant chemotherapy, coronary artery disease-CAD, atrial fibrillation or flutter, diabetes mellitus, hypertension, and renal failure) score was derived and validated. Low (0–3 points), medium (4–5 points), and high (=6 points) risk strata had three-year rates of HF or LV dysfunction of 16.2%,26%, and 39.5%, respectively. LV dysfunction can remain asymptomatic for a long time [13], but once symptomatic, the prognosis is among the worst in the HF population [14]. Moreover, chest radiation and cardiotoxic drugs (i.e., anthracyclines) have been noted to produce de novo AS via valve leaflet thickening, fibrosis, retraction, and calcification [15], but, at the present, the impact that they may have on AS progression has not been studied. Bravo-Jaimes et al. have found that patients with mild or

moderate AS and cancer are more likely to die before having AS progression, which is, in turn, associated with CAD and prevalent cyclophosphamide use [16].

Surgical valve replacement (SAVR), transcatheter valve implantation (TAVR), balloon valvuloplasty, and medical therapy are possible treatments for aortic valve stenosis, but when malignancy is present, the choice between these options must take into account the stage of the cancer and associated treatment, expected outcome, and comorbidities [17]. However, cancer-survivor patients with a confirmed remitted malignancy and evidence of severe AS, after an accurate multidisciplinary team evaluation with oncologists, an interventional cardiologist, and a heart surgeon, can be considered similar candidates to patients with no previous cancer history in terms of eligibility for aortic valve replacement. As recently reported by Płońska-Gościniak, patients with severe, pre-existing cancer and heart-valve disease should be managed according to the 2021 guidelines of the European Society of Cardiology and Cardiothoracic Surgery taking into consideration the cancer prognosis and patient preferences [18].

2. Pathophysiology

Clinical risk factors associated with AS development and progression mirror those associated with atherosclerosis, and because many are shared by cancer (advanced age, smoking, hypertension, hypercholesterolemia, obesity, metabolic syndrome, diabetes, and elevated lipoprotein (a) levels) prevalence and incidence rates of both disorders are rising simultaneously [19,20]. These common conditions, together with microbial and viral infections, allergen exposure, radiation, toxic chemicals, alcohol consumption, tobacco use, and other chronic and autoimmune diseases, induce inflammation [21]. It is now known that inflammation mediates all atherosclerosis stages, from initiation to progression and, ultimately, plaque unstabilization and thrombosis. Conditions such as hypertension, smoking, dyslipidemia, and insulin resistance all appear to trigger atherosclerosis, by promoting the expression of adhesion molecules by endothelial cells, allowing leukocyte attachment to blood vessel walls that normally resist their attachment. In recent decades, extensive factual and circumstantial evidence has shown several cancer types to be induced by infection or chronic inflammatory disease (e.g., human papillomavirus and cervical cancer, Helicobacter pylori and stomach cancer, and Epstein–Barr virus and lymphoma) [22]. As stated by Koene et al. [21], controlling cardiovascular disease risk factors can help reduce the risk of cancer. There is an urgent need to improve the health status of the population to reduce the prevalence of both diseases.

Although chronic inflammation is an indispensable feature of the pathogenesis and progression of both cardiovascular disease and cancer, additional mechanisms can be found at their intersection, such as nonmodifiable risk factors, including age, sex, and race/ethnicity, which are uncontrollable. There are obvious differences between male and female organs and hormonal fluctuations that influence both cardiovascular disease and cancer progression. Of all nonmodifiable risk factors, age is a steady independent variable with regard to cardiovascular disease and cancer, yet the associations between age and disease onset can be highly influenced by lifestyle parameters, such as diet, physical activity, body mass index, and smoking.

3. Treatment

Currently, the optimal strategy for the management of severe AS in patients with an active cancer is still unclear. Tailoring the correct and most optimal care for cancer patients with severe AS is complex. Asymptomatic patients with severe AS, in the absence of adverse prognostic features such as reduced LV ejection fraction, or symptoms appearance during an exercise stress test, are recommended for a watchful waiting approach, with regular and frequent follow-up and prompt intervention in case of clinical progression (i.e., symptoms). In this way, asymptomatic patients can proceed with their antineoplastic therapy without interruptions or delays. Medical treatment of hypertension and hyperlipidemia, according to current international guidelines, is recommended for

patients with severe AS. According to the 2020 American College of Cardiology Foundation/American Heart Association guidelines, adult patients with symptomatic severe AS (stage D), or with asymptomatic severe AS with LVEF <50%, or need of other cardiac surgery have an indication for aortic valve replacement (AVR). If the risk for a SAVR is high or prohibitive, decision-making focuses on TAVR or palliative care, depending on the life expectancy [23]. In the 2021 European Society of Cardiology Guidelines for The Management of Valvular Heart Disease, AVR is indicated in patients with symptomatic severe AS, except for those in whom the intervention is unlikely to improve quality of life or survival (due to severe comorbidities) or for those with concomitant conditions associated with survival <1 year [24]. In the past years, in patients with severe AS, priority was given to the treatment of neoplastic disease rather than the treatment of severe valvular disease. However, patients undergoing SAVR have shown markedly better survival, due to better resilience to anemia, infection/sepsis, and rapid volume changes from chemotherapy regimens or hypotension/volume loss during surgical procedures, not uncommon during cancer treatment. It must be said, anyway, that patients with severe AS are not excellent candidates for surgery mainly due to comorbidities that increase the estimated periprocedural morbidity and mortality [2]. Conflicting results come from reports where SAVR is performed before cancer surgery [25]. A fundamental problem of SAVR in cancer patients is that open surgery requires extracorporeal circulation. Among various other systemic effects, cardiopulmonary bypass can cause immunosuppression, increase inflammation (as demonstrated by a significant increase in TNF-alpha, Il-10, Il-6, Il-1, and TGF-beta), and worsen cancer outcomes. So, precisely because of the immunosuppressive effects, patients with hematologic cancers are at risk of having worse outcomes than those with solid tumors and better immune systems. However, the relationship between the use of extracorporeal circulation and cancer progression has not yet been clearly demonstrated. Among the comorbidities, we should also consider the vascular fragility that patients with active cancer can develop, and which can sometimes be caused by anticancer drugs or radiotherapy. In addition, cardiac surgery recovery times are longer, and this could lead to a delay and lengthening of the antineoplastic therapy times.

Even if is mandatory to consider each case individually (SAVR vs. TAVR), it is reasonable to conclude that TAVR, for cancer patients with severe AS, can more frequently be the best clinical choice by avoiding cardiopulmonary bypass and all its consequences. One of the biggest advantages of TAVR is its minimal invasiveness and, therefore, shorter recovery time. Moreover, thanks to the increasing access of cancer patients to TAVR, delays in cancer treatment have been significantly reduced from about 2 months after cardiac surgery to 2 weeks [26]. TAVR does not require a median sternotomy or cardiopulmonary bypass and can be performed under local anesthesia, which reduces the overall time required to complete the procedure, which benefits patients with malignancies. Manger et al. and Landes et al. reported that TAVR periprocedural mortality and major complication rates were equivalent in patients with and without active cancer [3]. Kojima et al. reported no difference in terms of complications between patients undergoing TAVR with and without active cancer [27]. However, despite the technical difficulties for open surgery that may be overcome by TAVR, major comorbidities may influence post-TAVR prognosis just as with SAVR [28]. To be eligible for TAVR, as mentioned before, cancer patients should have a prognosis of 1 year or greater. However, precise estimation of prognosis has always been very difficult in these patients, and even more so lately, thanks to the rapid expansion of new and innovative cancer therapies. A fundamental element to consider in choosing the most suitable type of intervention for the patient, in addition to the prognosis, is the stage of neoplastic pathology. Patients with a history of cancer, who are judged in remission by the oncology team, are usually eligible for TAVR. Patients with early cancer stages, who can safely receive oncologic treatment, could be easily considered for TAVR as soon as remission is confirmed. In other cases, performing TAVR before cancer treatment allows for radical oncologic treatment shortly after valve intervention [29]. Patients with AS and a localized cancer can be stabilized and TAVR can be considered after the exclusion of

metastatic disease. Patients with advanced disease stage, metastases, multiple comorbidities, and very short estimated survival may be candidates for balloon valvuloplasty as a "bridge to destination" surgery [30].

In the final stages of neoplastic disease, a more conservative approach aimed at improving quality of life during palliative treatment is preferred. A recent expert consensus issued by the Society for Cardiovascular Angiography and Interventions recommends aortic balloon valvuloplasty or TAVR for cancer patients with AS as either a palliative or cure for valvular disease, to improve quality of life or to facilitate appropriate treatment of cancer therapy. Unfortunately, due to the characteristics of advanced stage cancer patients, it is difficult to conduct large studies, limiting the quality of data to support this approach [30]. A small study from Schechter et al. of 65 cancer patients with severe AS found that valve replacement improves survival, regardless of the type of cancer or anticancer therapy, with TAVR being the most effective [26]. Nowadays, the majority of cancer patients diagnosed with severe AS undergo valve replacement before cancer treatment, with the large majority receiving TAVR more than SAVR. Despite the lower risk of TAVR complications, the literature is not univocal about what are the peri-procedural complications of TAVR that could cause a delay in cancer treatment and modify overall survival. A meta-analysis by Marmagkiolis et al. demonstrates a favorable post-TAVR short-term mortality and remarkable safety, with improved stroke and acute kidney injury (AKI) rates without increased bleeding and the need for new pacemaker implantation in cancer patients compared to controls [7]. Conversely, a meta-analysis from Bendary et al., reported higher rates of postprocedural pacemakers, without any difference in short-term mortality [31]. In a systematic review of Arocutipa et al., AKI occurred more frequently in patients with active cancer [32]. AKI is a very common complication of TAVR and can rate in up to 50% of procedures. Its origin is multifactorial: in addition to the iodinated contrast, bleeding and anemia, volume depletion, microembolisms, hypotension, or nephrotoxic drugs also contribute. Importantly, tumor type also plays a role in the risk of post-TAVR AKI. Thus, the decision to ultimately pursue TAVR is not an easy choice and involves a multidisciplinary and holistic approach to assessing the appropriateness of intervention. Recently, also in light of the study of Ullah et al. [33], which highlighted different outcomes between SAVR and TAVR based on the tumor location, our group proposed a detailed specific decision-making algorithm for the management of symptomatic severe AS in cancer patients, both active and in remission [34]. Specifically, in the case of active cancer, once it is ascertained that cancer-related life expectancy is >1 year, that cancer treatment is not feasible before AS treatment, and that cancer treatment can be delayed for at least 2 months, the decision-making process is comparable to cancer in remission. In this case, evaluation for the presence of high-risk features for SAVR and/or clinical conditions favoring TAVI TAVR is suggested. Where such conditions are not present, the choice between TAVR and SAVR rests in the judgment of the heart team, including the consideration that the tumor site can influence the management strategy and the personal patient choice (Figure 1).

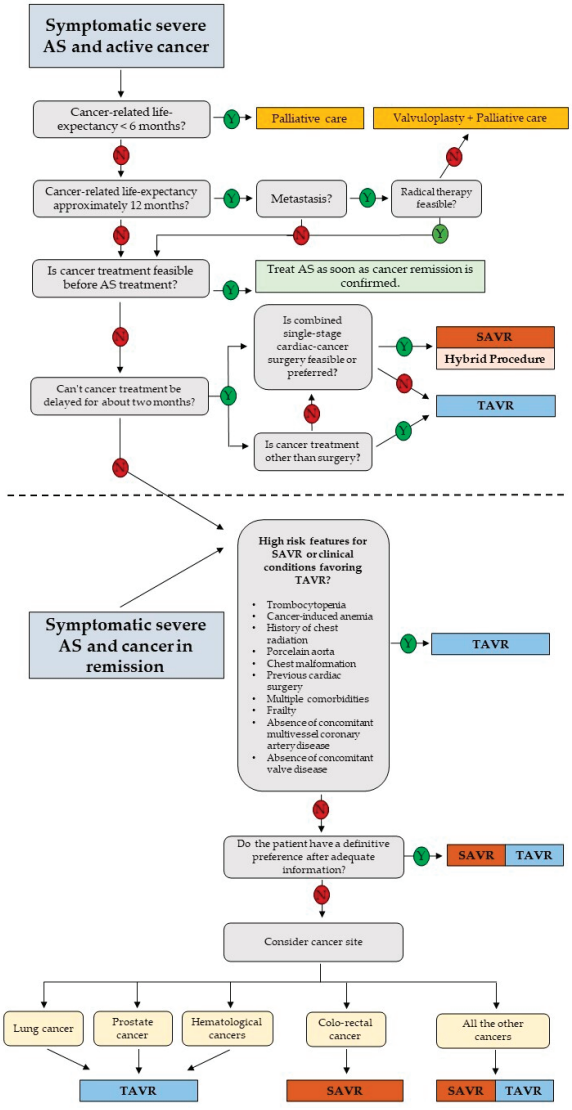

Figure 1. Proposed decision-making algorithm for the management of patients with severe aortic stenosis and cancer. Modified from ref. [34]. AS: aortic valve stenosis; SAVR: surgical aortic valve replacement; TAVR: transcatheter aortic valve replacement.

3.1. Radiation Therapy and Aortic Stenosis Treatment

An issue that should not be forgotten in the field of AS and cancer disease is the impact of radiotherapy on these patients. Chest radiation (C-XRT) is part of standard treatment protocols for various malignancies (i.e., lymphoma and breast, lung, and esophagus cancer) [35]. Radiation-induced valvular disease involves a degenerative process with early valve retraction resulting initially in regurgitation and, after, thickening and calcification of the valves leading to stenosis. Fibrosis and calcification of the mitral and aortic valves, especially surrounding structures including the annulus, subvalvular apparatus, and the aorto-mitral curtain [35], have been noted in patients who underwent C-XRT. Left-sided valves are most commonly affected probably due to the stimulus of higher pressure flows.

A dose-dependent toxic effect on the heart has been previously demonstrated, and immunobiological studies have shown, specifically, a dose-dependent effect of aortic valve fibrosis. It has been suggested that >30 Gy is considered a high dose of mediastinal radiation [36]. In terms of screening, the International Cardio-Oncology Society recommends obtaining a routine transthoracic echocardiogram in all patients who are planning to undergo thoracic radiation and every 5 years thereafter to screen for radiation-induced valvulopathy [37]. However, this recommendation has yet to be integrated into routine clinical practice. In a retrospective study of Hodgkin disease survivors with and without prior chest radiation, 6 of 49 (12%) patients who underwent C-XRT developed moderate or severe aortic regurgitation, mitral regurgitation, or AS, whereas only 1 of 29 patients without prior chest radiation developed more than mild AS, and 1 more than mild aortic regurgitation [38]. This patient group is plagued by a high mortality and presents unique challenges in surveillance and in balancing the risks and benefits of treatment [39]. Donnellan et al. [40] compared AS patients with prior exposure to C-XRT to a group with similar AS at baseline but no history of irradiation. Although the progression of AS was similar in both groups, significantly more patients in the C-XRT group underwent AVR for the development of symptoms (80% vs. 50%, $p < 0.001$) during a mean follow-up of 3.6 ± 2 years. Despite that, the C-XRT group had significantly higher long-term mortality than the comparison group. The decision-making of treatment modality for radiation-induced AS should be a multidisciplinary decision that is targeted for the patient's specific characteristics and needs, taking into account the complexity of anatomy and disease history. Patients with a prior history of radiation to the chest are considered to be at high risk for surgery for numerous reasons: the ascending aorta can be markedly calcified ('porcelain aorta') making cross-clamping difficult, the frequent need for associated mitral and coronary surgery, and the presence of pulmonary fibrosis, which correlate directly with mortality postoperatively [36]. Furthermore, C-XRT causes mediastinal adhesions and fibrosis that need to be dissected. They increase the risk of bleeding and poor wound healing. The treatment with debridement may increase the cardiopulmonary bypass time. Therefore, a history of prior chest radiation is now included in the STS risk score before a cardiac surgery, given its significant effect on surgical mortality [41]. A recent matched cohort study found that radiation was associated with a statistically significant increase in in-hospital mortality and a 6 year mortality after SAVR, compared with patients without a radiation history [42]. However, 61% of patients were undergoing SAVR with another concomitant procedure. Isolated SAVR has been shown to have better 5 year survival than combined procedures in patients with radiation-induced AS [43]. TAVR is an increasingly performed procedure and may be an important treatment avenue for patients with radiation-induced AS, taking into account the potential complications, such as fistulization and tissue rupture.

An accurate analysis with computed tomography angiography (CTA) evaluating aortic valve characteristics and size, access route, and degree of aortic calcifications for optimal TAVR planning is always mandatory, especially in this very high-risk setting [44]. Zafar et al. [45] showed in a 2020 systematic review and meta-analysis that TAVR was a safe option for patients with radiation-induced AS. Although current guidelines do not recommend TAVR in patients with a life expectancy of less than 1 year, many cancer survivors do not meet this timeline, and even those on active therapy are experiencing continued improvement in survival. There will, therefore, be a growing need to revisit the option and benefit of TAVR in cancer patients [7].

3.2. Aortic Stenosis, Coronary Artery Disease (CAD) and Mitral Regurgitation

Concomitant cardiovascular problems, such as CAD or mitral regurgitation (MR) should be promptly assessed before the treatment of AS. A surgical approach with combined valvular intervention and coronary revascularization may represent an extremely high-risk setting in fragile patients, such as cancer ones. Therefore, a percutaneous approach is reasonably the best option to adopt. As reported above, CTA of the aorta and the iliofemoral arteries is crucial for preprocedural planning, and the combination of coronary

computed tomography angiography (CCTA) may be useful for the exclusion of concomitant CAD in order to minimize invasive procedures in these high-risk patients [46]. In the case of concomitant significant CAD, American guidelines [23] recommend percutaneous coronary interventions (PCI) before TAVR in the case of left main disease and significant proximal CAD. Instead, European guidelines [24] suggest basing a decision according to the clinical presentation, coronary anatomy, and extent of myocardial risk. PCI concomitant with TAVR is recommended in patients at high risk of coronary obstruction by the prosthetic aortic valve (e.g., ostial lesion, low left main height, or a valve-in-valve implantation) or in patients in whom it is desirable to minimize dual antiplatelet therapy (DAPT) due to bleeding risk [47]. Regarding antithrombotic therapy, the standard treatment after TAVR is aspirin, while patients with an indication for oral anticoagulation therapy or DAPT should receive the specific treatment according to the pre-existent clinical indication without any concern to the valve procedure. This represents a concrete advantage, especially in patients with higher bleeding risk, such as cancer patients [48]. Furthermore, the progressively younger age of patient candidates for TAVR makes the possibility of reaccessing the coronary arteries increasingly important. Thus, further studies on increasing coronary reaccess after TAVR and the best timing of percutaneous coronary interventions in relation to TAVR are necessary.

The reported prevalence of moderate or severe mitral regurgitation (MR) in AS patients eventually undergoing SAVR or TAVR ranged between 19% and 33% [47,49]. Ruel et al. have found that AS patients with a functional MR $\geq$ 2+ and a left atrial diameter >5 cm, preoperative peak aortic valve gradient <60 mm Hg, or atrial fibrillation have a significantly higher risk of cardiac HF and persistent mitral regurgitation after AVR than other AS patients. Waisbren et al. [50], in their work, support a conservative, tailored approach to concomitant mitral surgery in patients presenting for the correction of AS who demonstrate functional regurgitation. Finally, in patients with severe MR, there is not enough experience to make recommendations about surgery versus combined or sequential TAVR and percutaneous mitral edge-to-edge repair. Currently, no consistent data is available about the coexistence of AS and MR and the related treatment in the subgroup of cancer patients.

3.3. Aortic Stenosis and Cardiac Amyloidosis

The prevalence of calcific AS and cardiac amyloidosis (CA) increases with age, and their association is, as expected, not uncommon in the elderly. Deposition of an amyloid substance, especially transthyretin, can involve any cardiovascular structure, including the aortic valve and myocardial walls, and it may contribute to the initiation and progression of AS, as well as progressive myocardial dysfunction. Until now, there is no recommendation or consensus on whether patients with AS should be systematically screened for CA [51]. In patients with coexisting CA, AS severity should be assessed according to the current guidelines [52]. Approximately 50% of patients with confirmed CA have a severe low-flow low-gradient AS with preserved LV ejection fraction, (the so-called "paradoxical low-flow, low-gradient pattern") [53], characterized by severe LV concentric remodeling, impairment of diastolic filling, left atrial remodeling and dysfunction, markedly reduced LV global longitudinal strain function, and right ventricular remodeling and dysfunction [4,25]. Additional imaging tests are required to differentiate a true-severe versus a pseudo-severe AS, such as dobutamine stress echocardiography noncontrast computed tomography in order to quantitate the aortic valve calcium burden. Until now, there is no randomized trial and no expert consensus that determines the best management of CA in patients with AS. There are very few data on the outcome and therapeutic management of patients with AS and concomitant CA. Most studies reported a high risk of mortality and nonimprovement in functional status following AVR in patients with severe AS and CA [53–55]. One study in a small number of patients (n < 30) suggests that the outcome of patients with AS and CA may be better with TAVR versus SAVR [53].

4. Conclusions

The cardio-oncology patient population has been increasing in recent years, requiring appropriate management strategies to improve quality of life and survival rate. The coexistence of significant aortic valve stenosis and cancer is relatively common and poses diagnostic and therapeutic dilemmas. Collaboration between cardiologists and oncologists is of primary importance to select the best treatment approach and optimize the timing of intervention. Further clinical trials and registry studies are needed to better appreciate outcomes in this complex setting.

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Abbreviations

AKI	acute kidney injury
AS	aortic stenosis
CA	cardiac amyloidosis
CAD	coronary artery disease
CCTA	coronary computed tomography angiography
CTA	computed tomography angiography
C-XRT	chest radiation therapy
DAPT	dual antiplatelet therapy
HF	heart failure
LV	left ventricular
MR	mitral regurgitation
PCI	percutaneous coronary interventions
SAVR	surgical valve replacement
TAVR	transcatheter valve replacement

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Review

Effects of Probiotics on Intermediate Cardiovascular Outcomes in Patients with Overweight or Obesity: A Systematic Review and Meta-Analysis

Frank Mayta-Tovalino [1,*], Carlos Diaz-Arocutipa [1], Alejandro Piscoya [1] and Adrian V. Hernandez [1,2]

[1] Unidad de Revisiones Sistemáticas y Meta-Análisis (URSIGET), Vicerrectorado de Investigación, Universidad San Ignacio de Loyola, Av. La Fontana 550, Lima 15024, Peru
[2] Health Outcomes, Policy and Evidence Synthesis (HOPES) Group, University of Connecticut School of Pharmacy, 69 N Eagleville Rd U-3092, Storrs, CT 06269, USA
* Correspondence: fmaytat@usil.edu.pe

Abstract: Background: Clinical trials evaluating the effect of probiotics on cardiovascular intermediate outcomes have been scarce in recent years. We systematically evaluated the efficacy of probiotics on intermediate cardiovascular outcomes in patients with overweight or obesity. Methods: We searched for randomized controlled trials (RCTs) in four databases (until August 2021) that evaluated the effects of probiotics versus controls on intermediate cardiovascular outcomes. The outcomes were body mass index (BMI), weight, systolic blood pressure (SBP), diastolic blood pressure (DBP), glucose, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels. Inverse variance random effects meta-analyses were used. The effects were reported as mean difference (MD), with their 95% confidence intervals (95% CI). The quality of evidence (QoE) was assessed with GRADE (grading of recommendations, assessment, development and evaluations) methodology. Results: A total of 25 RCTs were included (n = 2170), with a range of follow-up from two to six months. Probiotics likely reduced BMI (MD −0.27 kg/m^2, 95%CI: −0.35 to −0.19; 17 RCTs; I2 = 26%, QoE: moderate), as well as likely reduced weight (MD −0.61 kg, 95%CI: −0.89 to −0.34; 15 RCTs; I2 = 0%, QoE: moderate), and may have slightly reduce LDL (MD −4.08 mg/dL; 95%CI: −6.99 to −1.17; 9 RCTs; I2 = 87%, QoE: low) in comparison to the controls. However, probiotics had no effect on SBP (MD −0.40 mmHg; 95%CI: −5.04 to 4.25; 7 RCTs; I2 = 100%, QoE: very low), DBP (MD −1.73 mmHg; 95%CI: −5.29 to 1.82; 5 RCTs; I2 = 98%, QoE: very low), glucose (MD −0.07 mg/dL; 95%CI −0.89 to 0.75; I2 = 96%, QoE: very low), HDL (MD −1.83 mg/dL; 95%CI: −4.14 to 2.47; 14 RCTs; I2 = 98%, QoE: very low), or triglycerides (MD −3.29 mg/dL, 95%CI −17.03 to 10.45; 14 RCTs, I2 = 95%, QoE: very low) compared to control arms, and the evidence was very uncertain. Conclusions: In obese or overweight patients, BMI, weight, and LDL were lower in patients who received probiotics compared to those who received controls. Other lipids, glucose, and blood pressure were not affected by the probiotics.

Keywords: overweight; obesity; probiotics; meta-analysis

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1. Introduction

Probiotics are microorganisms with beneficial potential for human health. Currently, there is literature supporting the idea that intestinal probiotics may exert effects outside the digestive system, including regulating energy balance, cardiovascular benefits, and mechanisms associated with the absorption and breakdown of intestinal contents [1–4]. In addition, there are some probiotic strains that decrease the translocation of microorganisms and improve intestinal barrier function by reducing the release of proinflammatory cytokines [5,6].

Obesity has been identified as a critical global problem [7]. In the physiological context, obesity is complex because there are several intrinsic and extrinsic factors to be considered, as well as genetics, diet, and other nutrigenomic factors. Some studies have mentioned that

the gut microbiota has potential influence on the development of obesity. This is attributed to several mechanisms involving intestinal permeability and metabolic endotoxemia. In addition, a high-fat diet is closely associated with abdominal fat deposition and altered gut microbiota [8,9]. Furthermore, the intestinal microbiota is associated with the inflammatory process, insulin resistance, and type 2 diabetes mellitus. Intestinal microbiota is therefore considered a target in the treatment of diabetes and in the prevention of other cardiovascular diseases [9–11].

Recent literature has associated the development of obesity with an alteration in the intestinal microbiota (dysbiosis), which facilitates the storage of calories ingested in food. It is important to consider that there are certain intrinsic and extrinsic factors that can cause the imbalance of this intestinal ecosystem and which may lead not only to obesity, but also to the development of other alterations, such as insulin resistance. Some intervention studies show that oral administration of certain probiotics has a significant impact on some outcomes especially on body mass index (BMI) and weight control, suggesting a relationship between gut microbiota and body fat regulation [4–6,8]. For example, Firmicutes, Actinobacteria, Lactobacilli and Bifidobacterium are often related to these beneficial effects of probiotics [8–11].

We systematically evaluated the efficacy of probiotics on intermediate cardiovascular outcomes in patients with overweight or obesity.

2. Material and Methods

The PRISMA 2020 guidelines (Preferred Reporting Items for Systematic reviews and Meta-Analysis) were used for the writing and presentation of the present study [12]. In addition, this review was registered in PROSPERO (Prospective Registry of Systematic Reviews) (CRD42021264177).

2.1. Eligibility Criteria

We included studies that met the following inclusion criteria: (a) randomized controlled trials (RCTs) evaluating the effects of any dose and duration of probiotics on pre-defined intermediate cardiovascular outcomes; (b) a control group including milk, yogurt, maltodextrin, or placebo; and (c) evaluations adult patients ($\geq$18 years) who were overweight (BMI 25 to 30 kg/m^2) or obese (BMI > 30 kg/m^2). Excluded studies were observational studies, case series, and case reports and commentaries, systematic reviews, conference abstracts, and editorials. The population included in this meta-analysis had no systemic history of hypertension or diabetes.

2.2. Search Methods

Electronic searches were conducted on 2 August 2021 in the Scopus, Web of Science, PubMed, and Embase search engines. We elaborated the search strategy using free text words and MeSH terms for PubMed, then adapted them according to the other databases. There were no language or publication date restrictions (Supplementary Table S1).

2.3. Outcomes

Pre-defined intermediate cardiovascular outcomes were weight, BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), glucose, low-density lipoprotein (LDL), and high-density lipoprotein (HDL).

2.4. Selection and Data Collection of Studies

Study abstracts were downloaded to the Mendeley Reference Manager (Elsevier, Amsterdam, The Netherlands), and duplications were removed. The titles and abstracts were then independently reviewed by two authors (F.M.T. and C.D.A.). Subsequently, full-text articles were independently evaluated according to the selection criteria. All reasons for exclusion were recorded, and possible disagreements were resolved by consensus.

2.5. Data Extraction and Management

Data were extracted independently by two authors (F.M.T. and C.D.A.). An previously piloted extraction sheet was created in Microsoft Excel to record the author, year of publication, type of population (overweight, obese, both), mean age, proportion of diabetics and hypertensives, dose and duration of probiotic intervention, type of control, and outcomes for each intervention arm. Potential discrepancies were resolved by a third author (A.V.H.).

2.6. Assessment of Risk of Bias in Included Studies

To assess the risk of bias (RoB) of RCTs, the Cochrane RoB 2.0 tool was used [13]. Five domains of bias were assessed: randomization process, deviations from intended interventions, missing outcome data, outcome measurement, and selection of the reported outcome. Each bias domain was rated as "low," "high," or "some concerns." Each RCT was then rated as being at low RoB, if all domains were at low RoB, high RoB, if at least one domain was at high RoB, or with some concerns of bias, if at least one domain was identified at some concerns of RoB, and no domain was at high RoB. Two review authors (F.M.T. and J.B.O.) independently conducted the assessments, and disagreements were resolved by consensus.

2.7. Data Synthesis Methods

Inverse variance random-effects meta-analyses were performed for all outcomes. The between-study variance was estimated using the Paule–Mandel method [14]. Effect measures were described as mean differences (MD) and their 95% confidence intervals (CI). The heterogeneity of effects among RCTs was described using the I^2 statistic [15], with the following degrees: 0–30% (low), 30–60% (moderate), and >60% (high). Subgroup analyses by type of patient (overweight vs. obese vs. overweight/obese) and type of control (milk, yogurt, maltodextrin, or placebo) were conducted. The interaction test was considered statistically significant if the p-value was <0.10 [16]. The funnel plot and the Egger's test were used to evaluate publication bias, only if ten or more RCTs were available. The metabin and metacont functions of the meta package of R 4.1.2 (www.r-project.org) (accessed on 7 March 2022) were used for all analyses. A two-tailed $p < 0.05$ was considered statistically significant.

For the evaluation of the quality of evidence (QoE), the GRADE methodology was used [17], evaluating five domains: inconsistency, risk of bias, imprecision, indirectness, and publication bias. Finally, QoE was presented in summary tables (SoF) using GRADEpro GDT (https://gradepro.org/, accessed on 7 July 2022, McMaster University and Evidence Prime, Inc. 2020) European Union Seventh Framework Programme (FP7—HEALTH.2010.3.1-1—two stage).

3. Results

3.1. Selection of Studies

Of a total of 2851 abstracts, 1535 were available for evaluation, after removing duplicates. A total of 1374 records were excluded, and 161 full texts were further evaluated for inclusion. After excluding 136 studies after assessing populations, interventions, and outcomes that were out of the scope of our research question, we included 25 RCTs (n = 2170) in our study (Figure 1).

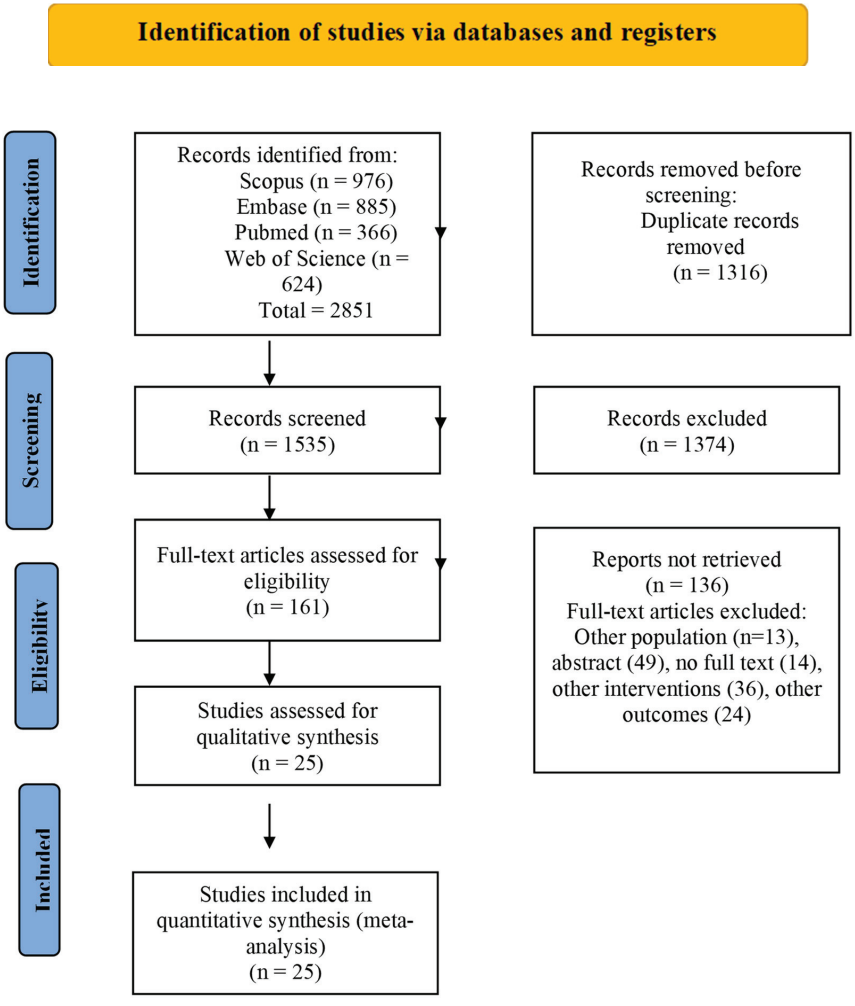


Figure 1. PRISMA Flow chart of the study selection process.

3.2. Characteristics of Included Trials

The studies included [18–42] in this systematic review were conducted in Denmark [18,20], Poland [19,30,40], USA [21], Iran [22,28,32,35,42], New Zealand [23,41], Korea [24–27], Malaysia [29], Japan [31], Indonesia [33], India [34,39], Canada [36], Russia [37], and Finland [38]. All RCTs had a follow-up period between 2 and 6 months. The study population was distributed across studies as follows: obesity [19–22,25–28,30–32,36–38,40–42], (n = 1603 patients), overweight [23,24,29,33–35,39], (n = 557 patients), and both overweight/obesity [18] (n = 70) (Table 1). The mean age range was between 28 and 68 years, there was no description of prevalence of diabetes, hypertension, or other cardiovascular diseases in individual RCTs. All included studies used probiotics of the bacterial genus (*Lactobacillus*, *Bifidobacterium*, *Streptococcus* and *Enterococcus*) [18–42]; control groups included placebo in 13 studies [19,21,22,24–27,29,30,34–37]; milk in four studies [18,23,31,33]; yogurt in two studies [28,42], and maltodextrin in six studies [20,32,38–41].

Table 1. Characteristics of included studies.

Author, Year	Country	Sample Size	Population	Age	Intervention	Control	Outcomes	Follow-Up (Month)
Agerholm-Larsen et al., 2020 [18]	Denmark	70	Overweight 10% and Obese 90%	38.6 ± 2.1	*Enterococcus faecium* (human species) and two strains of *Streptococcus thermophilus*. The subjects attended the department 3 days a week (mornings or afternoons) to consume 300 mL yogurt or one placebo tablet and to collect products for consumption at home.	The placebo milk product was of identical composition as the other milk products, but chemically fermented with an organic acid (delta-acid-lactone) instead of a living bacterial culture.	SBP	2
Banach et al., 2020 [19]	Poland	54	Obese	34.8 ± 9.2	*Lactobacillus acidophilus* LA-5 and *Bifidobacterium lactis* BB-12 strains	Hypocaloric diet without deliberates	BMI	3
Brahe et al., 2015 [20]	Denmark	58	Obese	61.4 ± 6.5	*L. paracasei* F19	Maltodextrin	Glucose, HDL	1.5
Culpepper et al., 2019 [21]	USA	103	Obese	51.2 ± 1.4	*Bacillus subtilis* R0179, *Lactobacillus plantarum* HA-119, *Bifidobacterium animalis subsp. lactis* B94	Placebo (potato starch)	Glucose	4.5
Hajippor et al., 2020 [22]	Iran	140	Obese	40.9 ± 6.7	*Lactobacillus Acidophilus La-B5* and *Bifidobacterium lactis Bb-12* (at levels of colony-forming 4 × 107)	Vitamin D	Cholesterol, HDL, LDL, Triglycerides.	2.5
Ivey et al., 2014 [23]	New Zeland	156	Overweight	68.4 ± 7.8	*Lactobacillus acidophilus La5* and *Bifidobacterium animalis* subsp lactis Bb12	Control milk (prepared by Harvey Fresh, Harvey, WA, Australia)	Glucose	1.5
Jung et al. 2015 [24]	Korea	95	Overwight	40.1 ± 1.4	*L. curvatus* HY7601 and *L. plantarum* KY1032	The same amountof powder that did not contain any probiotics.	BMI, glucose, SBP, DBP, cholesterol, LDL, HDL and triglycerides	3
Kim et al., 2017 [25]	Korea	60	Obese	37.9	*Lactobacillus curvatus* (*L. curvatus*) HY7601 and *Lactobacillus plantarum* (*L. plantarum*) KY1032	Placebo	BMI, weight	3
Lee et al., 2014 [26]	Korea	50	Obese		*Streptococcus thermophiles* (KCTC 11870BP), *Lactobacillus plantarum* (KCTC 10782BP), *Lactobacillus acidophilus* (KCTC 11906BP), *Lactobacillus rhamnosus* (KCTC 12202BP), *Bifidobacterium lactis* (KCTC 11904BP), *Bifidobacterium longum* (KCTC 12200BP), and *Bifidobacterium breve* (KCTC 12201BP).	Placebo	BMI, weight, cholesterol, triglycerides	2
Lim et al., 2020 [27]	Korea	95	Obese	46.4 ± 12.2	*L. sakei* CJLS03	Placebo	BMI, weight, glucose, cholesterol, HDL, LDL, triglycerides	3
Madjd et al., 2016 [28]	Iran	89	Obese	32.2 ± 6.9	*Lactobacillus acidophilus* LA5) and *bifidobacteria* (*Bifidobacterium lactis* BB12)	Simple yogurt	BMI, weight, HDL, triglycerides	3

Table 1. *Cont.*

Author, Year	Country	Sample Size	Population	Age	Intervention	Control	Outcomes	Follow-Up (Month)
Azlan et al., 2017 [29]	Malaysia	24	Overweight	28.0 ± 8.3	*Lactobacillus acidophilus*, *Lactobacillus lactis*, *Lactobacillus casei*, *Bifi dobacterium longum*, *Bifi dobacterium bifi dum*, and *Bifi dobacterium* infantis	Hexbio® B-Crobes Laboratory Sdn Bhd. Ipoh, Malaysia provided the MCP supplement and placebo samples.	Weight, glucose,	1
Majewska et al., 2020 [30]	Poland	50	Obese	55.2 ± 6.9	*Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W51, *Bifidobacterium lactis* W52, *Lactobacillus acidophilus* W37, *Lactobacillus brevis* W63, *Lactobacillus casei* W56, *Lactobacillus salivarius* W24, *Lactococcus lactis* W19, and *Lactococcus lactis* W58	Placebo	HDL, triglycerides	3
Naito et al., 2017 [31]	Japan	248	Obese	46.6 ± 1.1	*Lactobacillus casei* strain *Shirota* (LcS)	Placebo milk	BMI, weight, SBP, DBP, cholesterol, LDL, HDL, triglycerides	3
Narmaki et al., 2020 [32]	Iran	62	Obese	35.2 ± 5.7	*Lactobacillus acidophilus* (1.8×10^9 CFU/capsule), *Bifidobacterium bifidum* (1.8×10^9 CFU/capsule), *Bifidobacterium lactis* (1.8×10^9 CFU/capsule), *Bifidobacterium longum* (1.8×10^9 FU/capsule), *Lactobacillus rhamnosus* (1×10^9 CFU/capsule), *Lactobacillus reuteri* (1×10^9 CFU/capsule)	Magnesium stearate, and maltodextrin	BMI, weight	3
Rahayu et al., 2021 [33]	Indonesia	60	Overweight	44.0 ± 6.2	*Lactobacillus plantarum* Dad-13	Skim milk obtained from a local supermarket was used in the placebo group.	BMI, weight, cholesterol, HDL, LDL, triglycerides	3
Rajkumar et al., 2014 [34]	India	60	Overweight	49(40–60)	Bifidobacteria (*Bifidobacterium longum*, *Bifidobacterium infantis*, and *Bifidobacterium breve*), four strains of lactobacilli (*Lactobacillus acidophilus*, *Lactobacillus paracasei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Lactobacillus plantarum*), and one strain of *Streptococcus salivarius subsp. thermophilus*.	Omega 3	Cholesterol, HDL, LDL, triglycerides	1.5
Razmpoosh et al., 2019 [35]	Iran	70	Overweight	35.0 ± 10.0	*L. acidophilus* La5 and 1.79 106 CFU/g of B. lactis Bb12	Low energy diet	BMI, weight, SBP, DBP, cholesterol, HDL, LDL, triglycerides	2
Sanchez et al., 2014 [36]	Canada	153	Obese	37.0 ± 10.0	*Lactobacillus rhamnosus* CGMCC1.3724	Oligofructose and inulin	BMI, weight, glusoce, SBP, HDL	6

Table 1. *Cont.*

Author, Year	Country	Sample Size	Population	Age	Intervention	Control	Outcomes	Follow-Up (Month)
Sharafedtinov et al., 2013 [37]	Russia	40	Obese		*L. plantarum* TENSIA	Cheese	BMI, weight, SBP, DBP, HDL, triglycerides	1
Stenman et al., 2016 [38]	Finland	172	Obese	48.8 ± 10.5	*Bifidobacterium animalis* ssp. Lactis	Microcrystalline cellulose	BMI, weight, glucose, triglycerides	6
Sudha et al., 2019 [39]	India	92	Overweight	43.5	*Lactobacillus salivarius* UBLS-22, *Lactobacillus casei* UBLC-42, *Lactobacillus plantarum*, UBLP-40, *Lactobacillus acidophilus* UBLA-34, *Bifidobacterium breve* UBBr-01, and *Bacillus coagulans*	Maltodextrin	BMI, weight, cholesterol, LDL, HDL, triglycerides	3
Szulinska et al., 2018 [40]		110	Obese	55.1 ± 6.8	*Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W51, *Bifidobacterium lactis* W52, *Lactobacillus acidophilus* W37, *Lactobacillus brevis* W63, *Lactobacillus casei* W56, *Lactobacillus salivarius* W24, *Lactococcus lactis* W19, and *Lactococcus lactis* W58	Maize starch and maltodextrins	BMI, SBP, DBP	3
Tay et al., 2020 [41]	New Zeland	59	Obese	52.9 ± 8.7	*Lacticaseibacillus rhamnosus*	Microcrystalline cellulose and dextrose anhydrate	BMI, weight, glucose, cholesterol, LDL, HDL, triglycerides	3
Zarrati et al., 2018 [42]	Iran	60	Obese	36 ± 8.4	*Lactobacillus acidophilus* La5, *Bifidobacterium* BB12, and *Lactobacillus casei*	Conventional yogurts	BMI, weight	2

3.2.1. Risk of Bias and Quality of Evidence

Only three RCTs were scored as high risks of bias [19,31,33]. Two RCTs had a high risk of bias in the measurement of the outcome [31,33]; one RCT had a high risk of bias in the selection of the reported result domain [19]. Moreover, 11 RCTs had some concerns of bias [19,22,23,28–30,35–38,41] (Supplementary Figure S1) [20,21,24–27,32,34,39,40,42]. The outcomes SBP, HDL, and triglycerides had very low QoE; DBP and LDL had low QoE; and BMI, weight, and glucose had moderate QoE (Table 2).

Table 2. GRADE summary of findings table.

Outcomes	Anticipated Absolute Effects * (95% CI)		No of Participants (Studies)	Certainty of the Evidence (Grade)
	Risk with Control	Risk with Probiotics		
Body mass index follow-up: range 2 months to 6 months	The mean body mass index was 0.73 kg/m^2.	MD 0.27 kg/m^2 lower (0.35 lower to 0.19 lower)	1169 (17 RCTs)	⊕⊕⊕◯ Moderate [a]
Weight follow-up: range 2 months to 6 months	The mean weight was −1.07 Kg.	MD 0.61 Kg lower (0.89 lower to 0.34 lower)	998 (15 RCTs)	⊕⊕⊕◯ Moderate [b]

Table 2. *Cont.*

Outcomes	Anticipated Absolute Effects * (95% CI)		No of Participants (Studies)	Certainty of the Evidence (Grade)
	Risk with Control	Risk with Probiotics		
Systolic blood pressure follow-up: range 2 months to 6 months	The mean systolic blood pressure was −2.96 mmHg.	MD 0.4 mmHg lower (5.04 lower to 4.25 higher)	499 (7 RCTs)	⊕◯◯◯ Very low [c,d,e]
Diastolic blood pressure follow-up: range 2 months to 6 months	The mean diastolic blood pressure was −0.43 mmHg.	MD 1.73 mmHg lower (5.29 lower to 1.82 higher)	344 (5 RCTs)	⊕◯◯◯ Very Low [f,g,h]
Glucose follow-up: range 2 to 6 months	The mean glucose was −0.60 mg/dL.	MD 0.07 mg/dL lower (0.89 lower to 0.75 higher)	607 (9 RCTs)	⊕◯◯◯ Very Low [i,j,k]
Low-density lipoprotein follow-up: range 2 months to 6 months	The mean low-density lipoprotein was 1.39 mg/dL.	MD 4.08 mg/dL lower (6.99 lower to 1.17 lower)	562 (9 RCTs)	⊕⊕◯◯ Low [l,m]
High-density lipoprotein follow-up: range 2 months to 6 months	The mean high-density lipoprotein was 0.15 mg/dL.	MD 0.83 mg/dL lower (4.14 lower to 2.47 higher)	934 (14 RCTs)	⊕◯◯◯ Very low [n,o,p]
Triglycerides follow-up: range 2 months to 6 months	The mean triglycerides was −8.65 mg/dL.	MD 3.29 mg/dL lower (17.03 lower to 10.45 higher)	887 (14 RCTs)	⊕◯◯◯ Very low [q,r,s]

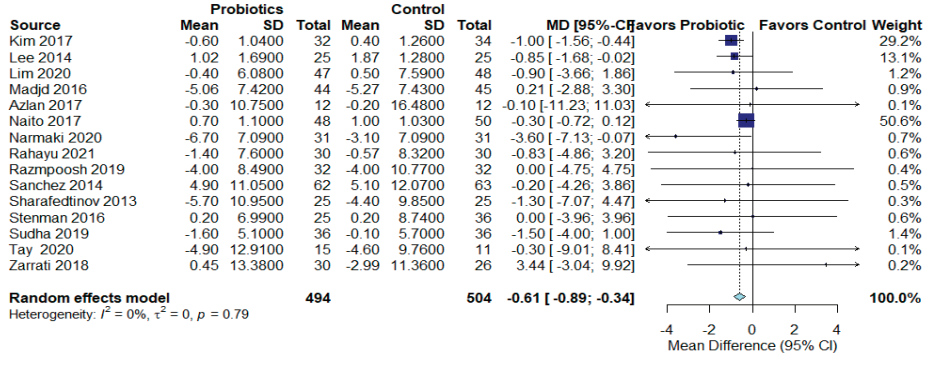
* The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI). The crosses are symbols marked according to GRADE methodology.
CI: confidence interval; MD: mean difference

GRADE working group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of the effect.

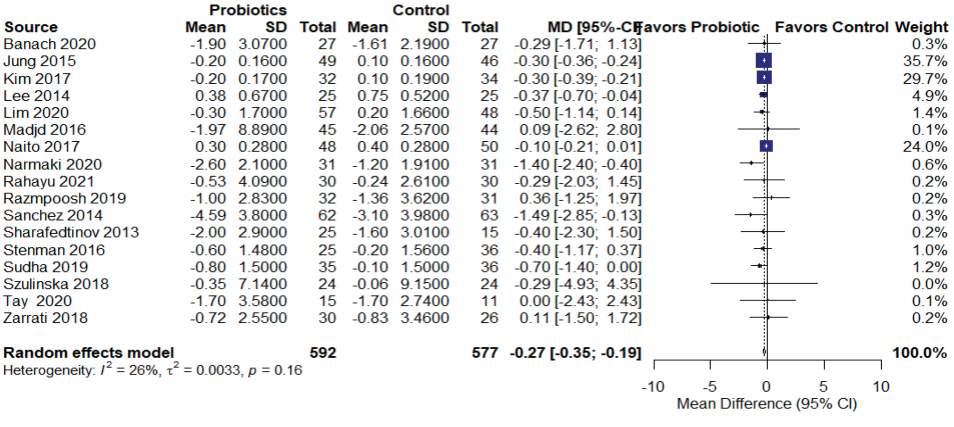
Explanation: **a.** RoB 2.0: Banach et al. had a high risk of bias in the selection of the reported results, Madjd et al. had some concerns in the deviations from intended interventions and the selection of the reported result, Naito et al. had high risk of bias in the measurement of the outcome and some concerns in the selection of the reported result. Rahayu et al. had some concerns in the deviations from intended interventions and in the selection of the reported result, Razmpoosh et al., Sanchez et al., Sharafedtinov et al., Stenman et al., Szulinska et al., and Zarrati et al. had some concerns in the selection of the reported results. **b.** RoB 2.0: Agerholm-Larsen et al., Naito et al., Razmpoosh et al., Sanchez et al., Sharafedtinov et al. and Szulinska et al. had some concern about the risk of bias in some of the dimensions evaluated. **c.** RoB 2.0: Naito et al. had a high risk of bias in the measurement of the outcome and some concerns in the selection of the reported result, Razmpoosh et al., Sanchez et al., Sharafedtinov et al., and Szulinska et al. had some concerns in the selection of the reported results. **d.** Inconsistency: I^2 = 100%. **e.** Imprecision: 95% CI of the effect was −5.04 to 4.25. **f.** RoB 2.0: Naito et al. had a high risk of bias in the measurement of the outcome and some concerns in the selection of the reported result. Razmpoosh et al., Sharafedtinov et al., and Szulinska et al. had some concerns in the selection of the reported results. **g.** Inconsistency: I^2 = 98%. **h.** Imprecision: 95% CI of the effect was −5.29 to 1.82. **i.** RoB 2.0: Azlan et al. had some concerns in the randomization process, deviations from intended interventions, and the selection of the reported results. Sanchez et al. and Stenman et al. had some concerns in the selection of the reported results. **j.** Inconsistency: I^2 = 96%. **k.** Imprecision: 95% CI of the effect was −0.89 to 0.75. **l.** RoB 2.0: Hajipoor et al. had some concern in the selection of the reported result, and Naito et al. had a high risk of bias in the measurement of the outcome and some concerns in the selection of the reported result. Rahayu et al. had some concerns in the deviations from intended interventions and in the selection of the reported result, and Razmpoosh et al., had some concerns in the selection of the reported results. **m.** Inconsistency: I^2 = 87%. **n.** RoB 2.0: Hajippor et al. had some concerns in the selection of the reported result. Madjd et al. had some concerns in the deviations from intended interventions and the selection of the reported result. Majewska et al. had some concerns in the selection of the reported result. Naito et al. had a high risk of bias in the measurement of the outcome and some concerns in the selection of the reported result. Rahayu et al. had some concerns in the deviations from intended interventions and in the selection of the reported result, and Razmpoosh et al., Sanchez et al., and Sharafedtinov et al., had some concerns in the selection of the reported results. **o.** Inconsistency: I^2 = 96%. **p.** Imprecision: 95% CI of the effect was −4.14 to 2.47. **q.** RoB 2.0: Hajippor et al. had some concerns in the selection of the reported result. Madjd et al. had some concerns in the deviations from intended interventions and the selection of the reported result. Majewska et al. had some concerns in the selection of the reported result. Naito et al. had a high risk of bias in the measurement of the outcome and some concerns in the selection of the reported result. Rahayu et al. had some concerns in the deviations from intended interventions and in the selection of the reported result, and Razmpoosh et al. and Sharafedtinov et al. had some concerns in the selection of the reported results. **r.** Inconsistency: I^2 = 95%. **s.** Imprecision: 95% CI of the effect was −17.03 to 10.45.

3.2.2. Effect of Probiotics on Weight and Body Mass Index

In 15 RCTs (n = 998) [25–29,31–33,35–39,41,42], probiotics likely reduces weight compared to the control group (MD −0.61 kg, 95% CI −0.89 to −0.34; I2 = 0%, QoE: moderate) (Figure 2a). In 17 RCTs (n = 1169) [19,24–26,28,31–33,35–42], probiotics likely reduced BMI compared to the control group (MD −0.27 kg/m^2, 95% CI −0.35 to −0.19; I2 = 26%, QoE: moderate) (Figure 2b).



(**a**)

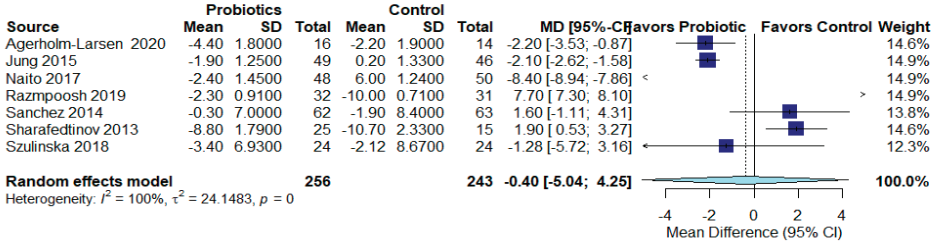


(**b**)

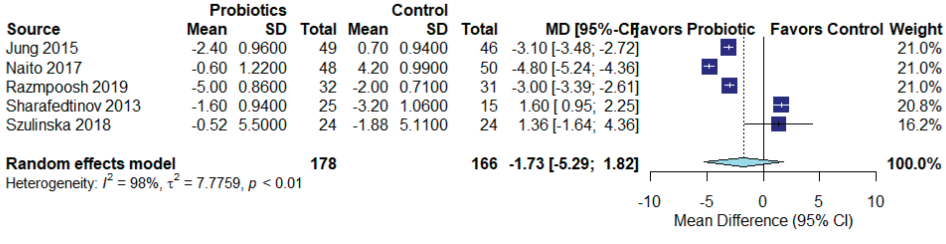
Figure 2. Effects of probiotics on (**a**) Weight in kg, and (**b**) BMI in kg/m^2.

3.2.3. Effect of Probiotics on Blood Pressure

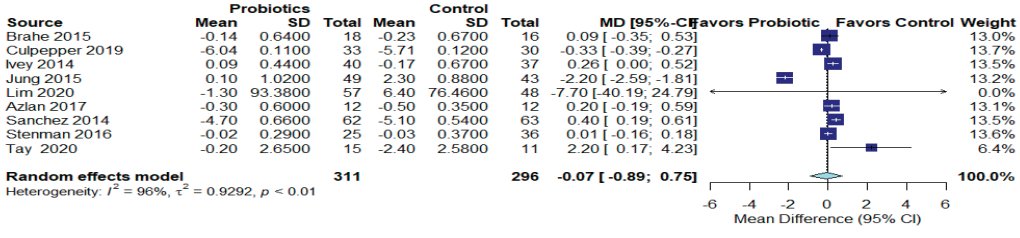
In seven RCTs (n = 499) [18,24,31,35–37,40], probiotics had no effect on SBP levels and controls (MD −0.40 mmHg; 95% CI −5.04 to 4.25; I2 = 100%, QoE: very low) (Figure 3a). In five RCTs (n = 344) [24,31,35,37,40], probiotics also had no effect on DBP levels and controls (MD −1.73 mmHg; 95% CI −5.29 to 1.82; I2 = 98%, QoE: very low) (Figure 3b). The evidence for SBP and DBP was very uncertain.



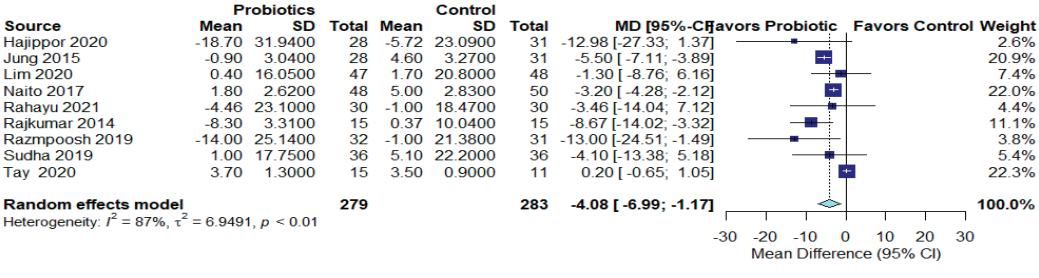
(**a**)



(**b**)

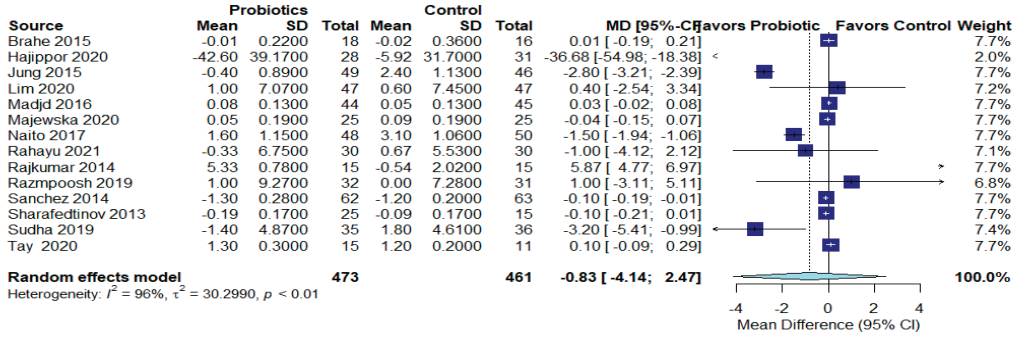


(**c**)

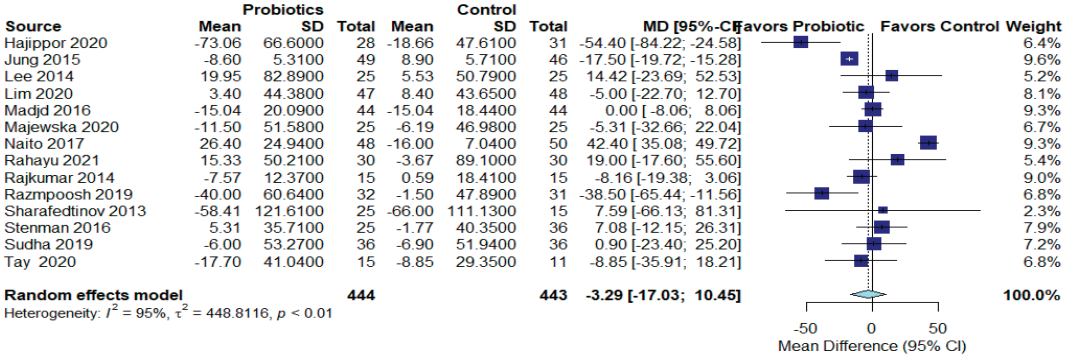


(**d**)

Figure 3. *Cont.*



(**e**)



(**f**)

Figure 3. Effects of probiotics on: (**a**) SBP in mmHg; (**b**) DBP in mmHg; (**c**) glucose (mg/dL); (**d**) LDL (mg/dL); (**e**) HDL (mg/dL); and (**f**) triglycerides (mg/dL).

3.2.4. Effect of Probiotics on Glucose

In nine RCTs (n = 607) [20,21,23,24,27,29,36,38,41] in overweight or obese patients, probiotics had no effect on mean glucose levels and controls (MD −0.07 mg/dL; 95%CI −0.89 to 0.75; I2 = 96%, QoE: very low) (Figure 3c), and the evidence was very uncertain.

3.2.5. Effects of Probiotics on Lipids

In 9 RCTs (n = 562) [22,24,27,31,33–35,39,41] in overweight or obese patients, those who received probiotics reduce LDL slightly compared to controls (MD−4.08 mg/dL; 95% CI −6.99 to −1.17; I2 = 87%, QoE: low) (Figure 3d). In 14 RCTs (n = 934) [20,22,24,27,28,30,31,33–37,39,41] in overweight or obese patients, probiotics had no effect on HDL levels and controls (MD −0.83 mg/dL; 95% CI −4.14 to 2.47 mg/dL; I2 = 96%, QoE: very low) (Figure 3e). In 14 RCTs (n = 887) [22,24,26–28,30,31,33–35,37–39,41] in overweight or obese patients, probiotics had no effect on triglyceride levels (mg/dL) and controls (MD −3.29 mg/dL; 95% CI −17.03 to 10.45; I2 = 95%, QoE: very low) (Figure 3f). The evidence was very uncertain for lipids.

3.3. Subgroup Analyses

Subgroup analyses by type of control showed that probiotics significantly reduced BMI when the control group was placebo and maltodextrin (p for interaction <0.01); for DBP, when the control group was milk (p for interaction <0.01); for cholesterol and LDL, when the control group was placebo and milk (p for interaction <0.01 for both); and for HDL only when the control was milk (p for interaction <0.01) (Figures S2–S9). Subgroup analyses

according to the type of patient showed that cholesterol and LDL were only reduced in overweight patients (p for interaction <0.01 and 0.03, respectively (Figures S10–S18). When analyzing the I^2 by subgroups, it was found that the percentage of heterogeneity remained very high in most of the outcomes analyzed. However, only BMI and weight decreased when analyzed by type of control and type of patient.

4. Discussion

In our systematic review and meta-analysis, we found that overweight and/or obese patients receiving probiotics had lower weight, BMI, and LDL levels in comparison to those receiving controls. Other intermediate outcomes, such as SBP, DBP, glucose, HDL and triglycerides levels, were not significantly different between the probiotic and control arms. QoE for BMI, weight, and glucose was moderate, while other outcomes had low and very low QoE. Finally, our subgroup analysis by type of control showed that probiotics reduced BMI, when the control group was placebo and maltodextrin. For DBP, when the control group was milk; for cholesterol and LDL, when the control group was placebo and milk; and for HDL, only when the control was milk. On the other hand, our subgroup analyses according to patient type showed that cholesterol and LDL were only reduced in overweight patients.

Probiotics are defined as compounds containing certain microorganisms that will improve the "good" microbiota of the human body, especially when administered in adequate doses and frequencies. These probiotics can have beneficial effects on health when consumed on regular basis [43–45]. They are usually found naturally, although there are also some foods to which these probiotics are added to generate better accessibility for the population. Probiotics could help reducing unwanted immune responses, thus preventing chronic inflammation [29,46,47]. Among the main benefits of probiotics in obese people, studies described that they could reduce body weight during a follow-up period of 6 to 12 months [48]. In addition, some studies have shown that the consumption of probiotics reduced lipid levels. Some strains of probiotics have also been found to reduce insulin resistance [34,49,50].

A previous meta-analysis by Park et al. [51] in 2015 showed no effect of probiotic intake on body weight (MD −1.77 kg; 95% CI −4.84 to 1.29 kg) and BMI (MD 0.77 kg/m^2; 95%CI −0.24 to 1.78 kg/m^2). The authors included four placebo-controlled RCTs (n = 9) until 28 December 2014, searched in PubMed, Cochrane Library, and EMBASE search engines, and this study was limited to research in humans, without language restriction, and considered randomized clinical trial type studies with probiotic supplementation intervention without restriction in dose or route of administration, and as a control, placebo or no intervention was used. Additionally, this study used the old 2011 RoB tool for RCTs and did not assess the QoE per GRADE.

In contrast, in 2018, Borgeraas et al. [52], using 15 placebo-controlled RCTs (n = 15), found that probiotic intake had a small important effect on body weight (MD −0.60 kg; 95% CI −1.19 to −0.01 kg) and BMI (MD −0.27 kg/m^2; 95%CI −0.45 to −0.08 kg/m^2). The authors searched RCTs until September 1, 2016, using Medline and EMBASE engines, and they included randomized controlled trials in adult patients who were overweight (BMI 25–29.9 kg/m^2) and obese (BMI $\geq$ 30 kg/m^2). However, they excluded patients with gastrointestinal disorders, as well as studies involving pregnant women. Other limitations included the absence of QoE evaluation and the assessment of a small set of outcomes. The discrepancy in the times established for the evaluation of the effect of probiotics could be an important factor influencing the results reported by these authors. Finally, the 2016 study by Nikbakht et al. [53] in RCTs (n = 18) found that the reduction in blood glucose in the probiotic group was a trivial effect (MD −0.18 mmol/L; 95%CI −0.37 to 0.00 mmol/L). The authors searched information until February 2015 in PubMed (MEDLINE), Scopus, Cochrane Library, and the Cumulative Index to Nursing and Allied Health Literature (CINAHL) search engines, and they evaluated randomized or quasi-experimental (non-randomized controlled trials), full-text, English-language, controlled trials investigating

the efficacy of probiotics or synbiotics in adults (age $\geq$ 18 years); they did not evaluate the certainty of the evidence.

Our meta-analysis had several strengths. First, we conducted a comprehensive search of four engines until August 2021, this being the most recent systematic review in contrast to those in previous studies. Second, we also used the most updated version of the RoB tool, the Cochrane RoB 2.0 tool, which was not used previously. Third, QoE per outcome was performed using GRADE methodology, which improved the understanding the strength of the probiotic effects. Fourth, we performed subgroup analyses in populations that may have differential effects of probiotics, in particular the type of patients and the types of controls. Finally, although we found statistically significant effects of probiotics on weight, BMI, and LDL levels, the absolute reductions are small and probably not clinically meaningful.

The present study had several limitations. First, a high heterogeneity of effects exists in regards to several outcomes, which may be due to methodological heterogeneity across the RCTs. We performed subgroup analyses by type of patient and type of control and found some effect of differences with respect to the main analyses, according to the type of controls. Second, most of the studies are from the Middle East and the East, so our findings may not be extrapolated to other populations, such as those in Latin America, North America, and Europe. Third, according to the GRADE methodology, QoE was very low for some intermediate outcomes due to the imprecision in some effects and the high risk of bias in some RCTs. Nonetheless, small important effects were found on weight and BMI, with moderate QoE. Fourth, clinical outcomes, such as mortality, myocardial infarction, and stroke, among others, were not evaluated in our systematic review, as these are scarce or not reported in the short period of follow-up of the included RCTs. Finally, the follow-up time across RCTs was short since most studies had an average follow-up of 6 months. Therefore, we could not evaluate the long-term effects of probiotics on our included studies.

5. Conclusions

In our systematic review of RCTs in overweight and obese populations, probiotics reduced BMI, weight, and LDL levels compared to placebo or other active controls, with a moderate to low quality of evidence. However, these effects were small in absolute terms and may not translate into clinically significant effects, indicating that the above findings should be taken with caution. Large RCTs with longer follow up are needed to evaluate the long-term effect of the intake of probiotics on intermediate cardiovascular outcomes and preferably, on clinical outcomes.

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Conflicts of Interest: The authors declare that there are no conflict of interest regarding the publication of this paper.

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Review

Implication of Lipids in Calcified Aortic Valve Pathogenesis: Why Did Statins Fail?

Mohamed J. Nsaibia [1], Anichavezhi Devendran [2], Eshak Goubaa [3], Jamal Bouitbir [4], Romain Capoulade [5] and Rihab Bouchareb [6,*]

[1] Department of Cell Biology and Molecular Medicine, Rutgers University, Newark, NJ 07103, USA; medscience82@gmail.com
[2] Department of Medicine, Cardiovascular Research Institute, The Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA; anichavezhi.devendran@mssm.edu
[3] Thomas Jefferson University East Falls, Philadelphia, PA 19144, USA; shkgoubaa@gmail.com
[4] Department of Pharmaceutical Sciences, Division of Molecular and Systems Toxicology, University of Basel, 4056 Basel, Switzerland; jamal.bouitbir@unibas.ch
[5] L'institut Du Thorax, Nantes Université, CNRS, INSERM, F-44000 Nantes, France; romain.capoulade@univ-nantes.fr
[6] Department of Medicine, Division of Nephrology, The Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
* Correspondence: rihab.bouchareb@mssm.edu or rihabdahou@gmail.com; Tel.: +1-(212)-241-8471

Abstract: Calcific Aortic Valve Disease (CAVD) is a fibrocalcific disease. Lipoproteins and oxidized phospholipids play a substantial role in CAVD; the level of Lp(a) has been shown to accelerate the progression of valve calcification. Indeed, oxidized phospholipids carried by Lp(a) into the aortic valve stimulate endothelial dysfunction and promote inflammation. Inflammation and growth factors actively promote the synthesis of the extracellular matrix (ECM) and trigger an osteogenic program. The accumulation of ECM proteins promotes lipid adhesion to valve tissue, which could initiate the osteogenic program in interstitial valve cells. Statin treatment has been shown to have the ability to diminish the death rate in subjects with atherosclerotic impediments by decreasing the serum LDL cholesterol levels. However, the use of HMG-CoA inhibitors (statins) as cholesterol-lowering therapy did not significantly reduce the progression or the severity of aortic valve calcification. However, new clinical trials targeting Lp(a) or PCSK9 are showing promising results in reducing the severity of aortic stenosis. In this review, we discuss the implication of lipids in aortic valve calcification and the current findings on the effect of lipid-lowering therapy in aortic stenosis.

Keywords: aortic valve; lipids; statins; Lp(a); PCSK9

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1. Introduction—Calcific Aortic Valve Disease (CAVD)

Calcific Aortic Valve Disease (CAVD) is the most prevalent heart valve disorder in developed countries; it is a progressive fibrocalcific disease [1,2]. Different risk factors, such as age, male gender, hypertension, metabolic syndrome, diabetes, and bicuspid aortic valve (BAV), have been associated with CAVD [1]. The pathophysiology of CAVD is complex, involving several pathological processes including lipid retention, oxidation, chronic inflammation, fibrotic remodeling, and calcification. Histological analysis of explanted calcified aortic valves revealed the presence of osteoblast-like cells, chondrocytes [3], and inflammatory cells [4,5]. Inflammation and growth factors play an important role in promoting extracellular matrix (ECM) synthesis and in triggering the activation of the osteogenic program in valvular interstitial cells (VICs) [6–9]. Indeed, proteomics analysis of explanted human pathological aortic valves showed significant disruption of ECM components [7]. Proteoglycans, such as biglycan and decorin, play a major role in lipid retention and modification in CAVD [8,10,11]. In this regard, biglycan, which is an endogenous agonist of Toll-like receptor 2 (TLR2), has been shown to promote inflammation and the osteogenic transdifferentiation of VICs [11].

Moreover, genome-wide association and Mendelian randomization studies have pointed out the association of low-density lipoproteins (LDLs) and lipoprotein(a) (Lp(a)) with the development of CAVD [12,13]. In this regard, several mechanistic studies have confirmed the implication of Lp(a) and its lipid content in the activation of the osteogenic program of VICs [14–16].

Although lipids seem to be a key factor in the pathogenesis of CAVD, three randomized clinical trials (RCTs) failed to demonstrate any significant benefit of LDL lowering with statins on the progression of aortic stenosis [17–19]. Furthermore, plasma levels of Lp(a) are not significantly modified by statin therapy [20]. Therefore, there is significant interest in targeting Lp(a) levels with novel therapeutic agents, such as antisense oligonucleotides, to slow the progression of CAVD [21–23]. Furthermore, Langsted et al. (2016) found that patients with a PCSK9 loss-of-function mutation (PCSK9 R46L) have lower serum LDL and Lp(a) levels and a lower risk of calcific aortic stenosis based on data from 103,083 people in the Copenhagen General Population Study [24]. Herein, we examine the implication of lipids in CAVD. In addition, we have placed special emphasis on statins' failure to reduce the progression of aortic stenosis.

2. Role of Lipoprotein(a) and Oxidized Phospholipids in CAVD

2.1. The Implication of Nitric Oxide (NO) Activity in CAVD

Endothelial nitric oxide synthase (eNOS) uncoupling appears to be one central mechanism during early-stage disease and contributes to CAVD progression [25]. Indeed, NO synthesis and signaling are markedly affected by the oscillatory shear stress in the endothelium lining the calcification-prone fibrosa compared with the disease-resilient ventricularis [25]. In vivo studies have shown that a lack of eNOS in mice promotes a CAVD-like phenotype [26], whereas restoring paracrine NO signaling blunts VIC-driven calcification in different experimental models [27]. Furthermore, studies have also reported that NO maintains valvular homeostasis through guanylyl cyclase/cGMP- and NOTCH1-dependent mechanisms [28,29]. More recently, Majumdar et al. (2021) deepened mechanistic insights by showing that valvular endothelial cell (VEC)-derived NO rescues calcification by an S-nitrosylation-mediated mechanism in porcine aortic valve interstitial cells. The alteration of nitric oxide (NO) signaling and activity in CAVD induces reactive oxygen species generation, which may promote the production of oxidative lipid species, triggering inflammation signaling activation in the aortic valve [27]. Targeting eNOS may open new therapeutic avenues to restore the paracrine homeostasis of endothelial/valve interstitial cells [29].

2.2. The LPA Gene Locus and CAVD

The *LPA* gene locus determines circulating Lp(a) levels primarily, with no significant dietary or environmental influences (Thanassoulis et al., 2013). The *LPA* gene is located on chromosome 6 (6q25.3-q26) and has a high degree of homology with the plasminogen gene (PLG). The *LPA* locus is complex, with copy number variants (CNVs) in the region encoding the kringle IV type 2 (KIV2) domain, which is inversely related to Lp(a) levels in the blood [30]. Genome-wide association (GWA) studies and Mendelian randomization (MR) analyses have underlined a causal association between a common gene variant, rs10455872, located in the *LPA* gene locus and CAVD [12,31].

However, a recent GWA study did not find an association between *LPA* and CAVD in patients with a congenital bicuspid aortic valve [31]. A large meta-analysis conducted on 1797 CAVD cases and 131,932 controls revealed that carriers of rs10455872 have a 1.66-fold higher risk of developing CAVD [32]. Furthermore, studies showed that genetically determined lower levels of Lp(a) were associated with a 37% reduced risk of CAVD [33]. These findings suggest that lowering Lp(a) levels and/or blocking the specific pathways by which Lp(a) promotes CAVD could lead to therapies that slow the progression of CAVD.

2.3. Lp(a), a Major Carrier of Oxidized Phospholipids (Ox-PL), Is a Risk Factor for Aortic Stenosis

Lp(a) is composed of low-density lipoprotein (LDL)-like particles in which apolipoproteinB-100 (apoB) is covalently bound by a single disulfide bond to the glycoprotein apolipopr-

otein(a) (apo(a)) [30,34]. Apolipoprotein(a) is highly polymorphic, with a variable number of (KIV2) domains. The copy number variant of KIV2 domains determines the length of the lipoprotein, and it is inversely related to the level of Lp(a) in circulation. Studies have underlined the high-content oxidized phospholipids (Ox-PL) in Lp(a) particles [35]. Ox-PL binds to the KIV type 10 domain and is thus ferried, as a cargo, by Lp(a). Hence, Ox-PLs carried by Lp(a) may contribute to endothelial dysfunction, inflammation, and the expression of genes with pro-calcifying properties [35].

Ox-PL has been established as a causal risk factor for AS in several genetic and population studies [12,31,36]. The genetically determined level of Ox-PL linked to apo(a) (Ox-PL-apo(a)) increases the risk of CAVD 1.09-fold [37]. Interestingly, clinical imaging with 18F sodium fluoride positron emission tomography or computed tomography has revealed the presence of aortic valve micro-calcification in individuals with elevated Lp(a) prior to the development of clinical manifestations of CAVD (Despres et al., 2019). Together, these studies thus highlight that Lp(a) and its cargo, Ox-PL, are involved in the development of CAVD.

2.4. Lipid Oxidation Promotes Calcific Aortic Stenosis

Increasing evidence suggests that the infiltration of lipoproteins into the aortic valve plays a central role in promoting inflammation, which, in turn, might induce the activation of the osteogenic program in VICs [14,38]. Histological analysis of explanted calcified aortic valves has revealed the presence of several apolipoproteins (apo), such as apoB, apoE, apoA1, apolipoprotein E, and apo(a) [39–41]. Furthermore, there is an association between the level of Ox-LDL, the degree of inflammation, and fibrocalcific remodeling [42,43].

In vitro studies showed that Ox-LDL and several oxidized phospholipid (Ox-PL) species carried by the Lp(a) fraction promote the calcification of VICs [44]. In turn, lipoprotein-associated phospholipase A2 (Lp-PLA2) transforms Ox-PLs into lysophosphatidylcholine (LysoPC), which acts as a reactive metabolite that promotes the mineralization of VICs [45]. Immunohistochemical studies have highlighted the co-localization of Lp-PLA2 with Ox-LDL, suggesting that Lp-PLA2 could be transported by lipoproteins into the aortic valve [45–47]. Together, these studies suggest that the accumulation of oxidized lipids triggers osteogenic response activation in VICs [35] (Figure 1).

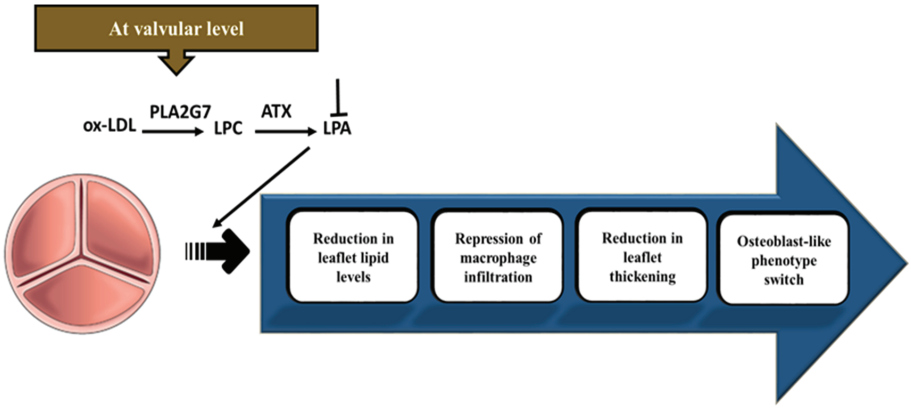

Figure 1. The implication of Ox-LDL in the calcification of the aortic valve. The infiltration of Ox-LDL into the aortic valve activates inflammation and, consequently, the release of PLA2G7 from macrophages, leading to the production of LysoPC. The activation of autotaxin leads to the preproduction of LPA, which amplifies the inflammation and the activation of the osteoblastic-like phenotype switch.

3. Autotaxin (ATX)–Lysophosphatidic Acid (LysoPA) Axis Mediates Mineralization of the Aortic Valve

Autotaxin (ATX) is a member of the ecto-nucleotidase family of enzymes encoded by the ENPP2 gene [48]. It was initially isolated from melanoma cell lines and was identified as a motility factor [49]. ATX is a secreted glycoprotein that hydrolyzes lysophosphatidylcholine (LysoPC) into lysophosphatidic acid (LysoPA). LysoPA is an active metabolite with potent and diverse biological properties. It promotes cell motility, inflammation, calcification, and fibrosis [49]. It is believed that the majority of circulating LysoPA is derived from ATX [50]. According to Bouchareb et al. (2015), ATX is likely transported into the aortic valve by Lp(a), and it is also secreted by VICs in response to inflammatory stimuli [15]. Indeed, ATX activity is enriched in an isolated fraction of Lp(a). Moreover, binding assay analysis, using human purified Lp(a), confirmed the physical association between Lp(a) and ATX [51]. In vitro inhibition of ATX prevented the mineralization of VICs induced by LysoPC, suggesting that LysoPA is the mediator promoting the activation of the osteogenic program in VICs [15]. Of interest, ATX expression and activity were increased in human explanted pathological aortic valves [15]. To this effect, stimulation of VICs with LysoPC and Ox-PLs treatment induces the expression of ATX [15,38]. Moreover, the administration of LysoPA to LDR$^{-/-}$ apoB$^{100/100}$ IGFII mice increased the osteogenic activity in the aortic valve and accelerated the development of CAVD [15,38]. Following a series of in-depth investigations, it has been shown that ATX and LysoPA promote aortic valve inflammation and mineralization through the activation of the NF-κB/bone morphogenetic 2 (BMP2) pathway [15,38]. In this regard, a significant interaction term was found between ATX activity and Lp(a) level [16]. Together, these studies indicate that ATX is carried by Lp(a) and is also secreted by VICs, increasing LysoPA levels and, therefore, stimulating inflammation.

More recently, Bouchareb et al. (2019) also showed that activated platelets promote VIC mineralization in vitro through the activation of ATX. In addition, ATX activity was higher in platelets from patients with CAVD compared to control patients (Bouchareb et al., 2019).

ATX promotes inflammation and the osteogenic transdifferentiation of VICs through the production of LysoPA, which is a small lipid derivative acting on G-protein coupled receptors with various biological functions [38]. In vitro studies have shown that oxidized LDL (Ox-LDL) induces the mineralization of VIC cultures, whereas treatment with an antagonist of LPAR1 prevents this effect [38]. The same study has also highlighted the overexpression of LPAR1 in human calcified aortic valves. In vitro studies using human VICs showed that lysoPA treatment stimulates the expression of the bone morphogenetic protein (BMP2) via the activation of the NFkB pathway. The promoter region of BMP2 contains NFkB-responsive elements and LysoPA promotes the phosphorylation of p65 on serine 536 (p65 S536). Of particular interest, phosphorylated p65 S536 was recruited to the promoter of BMP2 to activate BMP2 gene expression [38]. The pharmacological inhibition of LPAR1 with Ki16425 in LDLR$^{-/-}$ apoB $^{100/100}$ IGFII mice reduced the progression of CAVD and downregulated the expression of BMP2 in aortic valve cusps [38].

Lastly, Mkannez et al. (2018) [52] have recently underlined that the expression and the enzymatic activity of PLPP3 (also known as PPAP2B), a phospholipid phosphatase that inactivates LysoPA, were decreased in human calcified aortic valves compared to controls. Consistently, aortic valves with lower expression of PLPP3 had an increased level of LysoPA [52]. Furthermore, the knockdown of PLPP3 exacerbated the LysoPA-induced expression of BMP2 and consequently simulated in vitro VICs' mineralization [52].

4. The Implication of apoC-III in the Calcification of the Aortic Valve

Metabolic syndrome is described as a dysmetabolism related to insulin resistance and visceral obesity, which leads to a pro-inflammatory and pro-thrombotic state [53]. This syndrome has been associated with the increased incidence and progression of aortic valve calcification [54] and hemodynamic progression of aortic valve stenosis [55], making the visceral obesity-related perturbations a potential target to reduce the development and progression of CAVD.

One of the main features of metabolic syndrome is hypertriglyceridemia. The apolipoprotein C-III (apoC-III) is associated with elevated triglyceride levels. As opposed to other apolipoproteins, such as apo(a) or apoB, multiple particles of ApoC-III are carried by all lipoproteins [56,57]. This finding supports the use of ApoC-III as a potential biomarker, independent of the other lipid factors, and a potential therapeutic target. Indeed, ApoC-III's circulating levels have been linked to an increased risk of cardiovascular events, and targeting this apolipoprotein is a promising way to lower the risk for these patients [58–61].

ApoC-III is described as a multifunctional protein, playing a role in the metabolism of several lipoproteins, glucose homeostasis, endothelial cell dysfunction, inflammation, and the coagulation cascade, and in increasing lipoprotein affinity to the extracellular matrix (especially proteoglycans) [57]. Interestingly, all these features have also been associated with the development and/or progression of CAVD and support a potential detrimental action of this apolipoprotein in the pathogenesis of CAVD. This was further studied in a post-hoc analysis of the ASTRONOMER trial, where the Lp(a) content in ApoC-III has been related to faster CAVD progression [61]. As previously stated, this study provides evidence that, in addition to Lp(a) plasma levels, the content of this lipoprotein would be of great interest in understanding the mechanisms leading to aortic valve calcification and the development of aortic stenosis. Further studies focused on these aspects are needed and, by compiling data, would provide mechanistic evidence to target these lipoproteins in the context of CAVD.

5. The Effect of Lipid-Lowering Therapy in Aortic Stenosis

5.1. Rationale of Statins

Statins or the hydroxymethylglutaryl-CoA reductase (HMG-CoA) inhibitors are considered a potent therapeutic strategy in patients with atherosclerotic plaques. Statins are unquestionably well documented for their lipid-lowering effects [62,63]. Indeed, statins have demonstrated an improved survival rate in subjects with atherosclerotic coronary heart disease (CHD) [23]. They target the HMG-CoA reductase that catalyzes the switching of HMG-CoA to mevalonic acid, which abolishes the production of cholesterol [64]. The inhibition of HMG-CoA reduces cholesterol levels through the up-regulation of hepatocyte-LDL receptors, thus increasing the uptake of circulating LDL-cholesterol into the hepatocytes and subsequently decreasing LDL levels [65].

5.2. Statins' Mechanism of Action

Statins obstruct HMG-CoA reductases, the pivotal catalytic enzyme in the cholesterol biosynthesis pathway (Figures 2 and 3). This enzyme predominantly regulates the conversion of HMG-CoA synthases to mevalonic acid and, hence, in the course of action, manifests a decline in the plasma LDL levels [62]. Primarily, the lipid-lowering pleiotropic effects of statins are supposedly based on hampering the production of significant isoprenoid intermediates such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate in the cholesterol biosynthesis pathway [66–69]. In addition, they were proven to block the destabilizing effects of mevalonate on nitric oxide synthase (NOS)-mRNA in human endothelial cells (ECs), thereby resulting in the increased synthesis and function of the NOS enzyme [23].

5.3. Statin-Mediated Lipid-Lowering Therapeutic Approaches to Target CAVD: Past, Present, and Future Prospects

The existence of similarities in the pathophysiology of atherosclerosis and aortic stenosis has led the clinical and research community to consider statins as a treatment to decelerate the progression or to reduce the severity of aortic stenosis. Indeed, studies are showing the implication of Ox-LDL in the activation of osteogenic transition in calcified aortic valves [5,16,38,45]. In light of the facts presented, these results elicited considerable interest among the scientific community that eventually paved the way to the initiation of numerous randomized controlled clinical trials to elucidate the effect of a lipid-lowering

therapeutic regimen in aortic stenosis. Clinical studies, however, found no benefit of statins in terms of the hemodynamic progression or disease severity of aortic stenosis [17,18]. In the Simvastatin and Ezetimibe in Aortic Stenosis (SEAS) trial, patients were randomized to receive simvastatin 40 mg plus ezetimibe 10 mg, or a placebo [18,70]. The drug combination significantly lowered LDL levels compared to the placebo. However, no effect on the progression of aortic stenosis was observed [70]. To further complicate the situation, another meta-analysis study by Teo et al. [71] using randomized placebo-controlled clinical trials on 2344 patients reported no differences in clinical outcomes between the placebo and the treatment group. As a result of these negative outcomes of the conducted trials, the American Heart Association/American College of Cardiology and the European Society of Cardiology guidelines together do not endorse the use of statins for the treatment of CAVD [72,73].

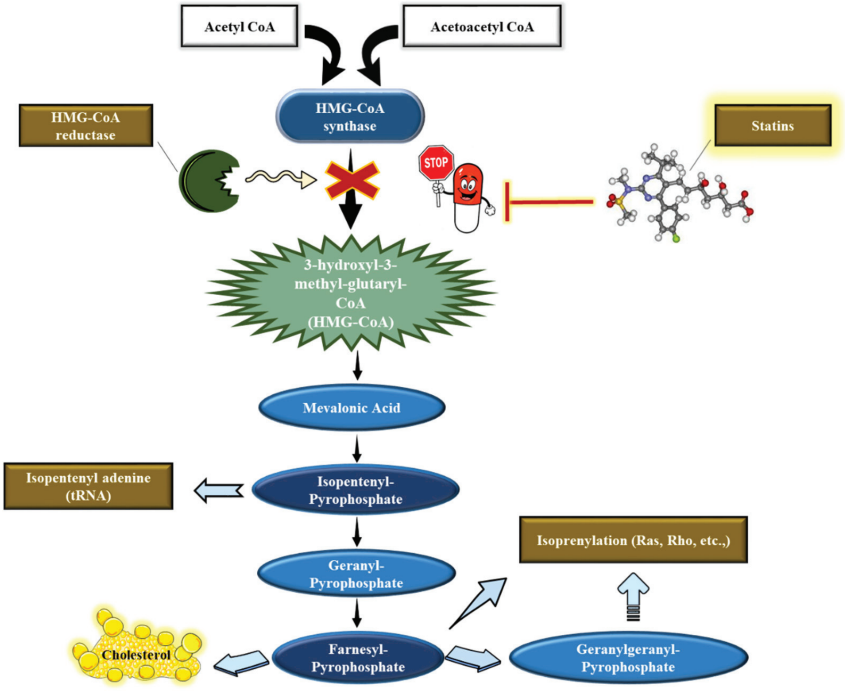

Figure 2. Statins' mechanism of action. Statins inhibit the HMG-CoA reductase to block the synthesis of mevalonic acid and, consequently, the production of cholesterol.

Nonetheless, the Aortic Stenosis Progression Observation: Measuring Effects of Rosuvastatin (ASTRONOMER) study showed an association between elevated levels of oxidized phospholipids and lipoprotein(a) (Lp(a)) in patients with accelerated hemodynamic progression of CAVD [74]. These findings support the hypothesis that Lp(a) mediates AS progression through its binding to Ox-PL [15,37,74]. These findings paved the way to the implementation of randomized clinical trials focusing on Lp(a)-lowering therapy in mild-to-moderate CAVS patients with elevated Lp(a) plasma levels (Figure 4).

Two randomized, double-blind, placebo-controlled trials are ongoing. In patients with elevated Lp(a), phase 1 and 2 trials demonstrated the tolerability, safety, and beneficial effect of lowering Lp(a) concentrations with IONIS-APO(a)Rx, an oligonucleotide targeting apolipoprotein(a). This therapeutic agent was found to reduce circulating Lp(a) by 80% (Koren et al., 2022; Tsimikas et al., 2015; Viney et al., 2016). A phase 3 clinical trial (Lp(a) HORIZON trial; NCT04023552) is now underway to assess the impact of this treatment on

clinical outcomes in patients with established cardiovascular disease and elevated Lp(a) plasma levels.

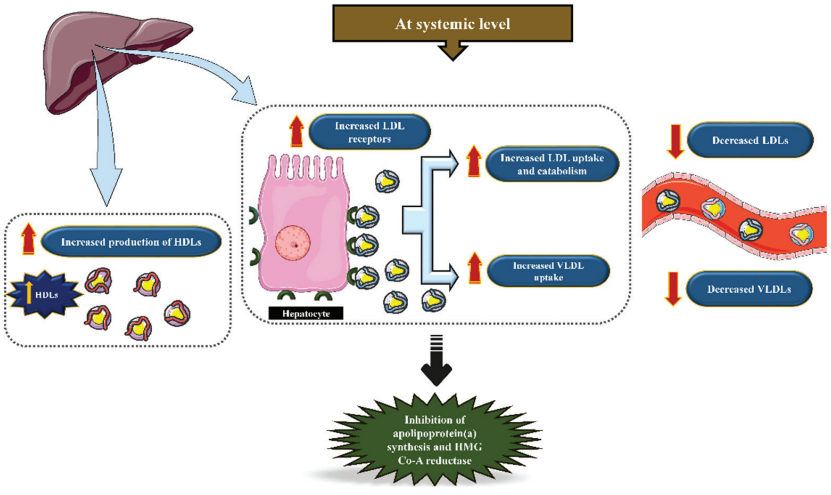

Figure 3. The effects of statins at systemic levels. Statins increase the expression of LDL receptors on hepatocytes to increase LDL uptake and catabolism, leading to a systemic decrease in LDLs, vLDLs, and the production of HDL.

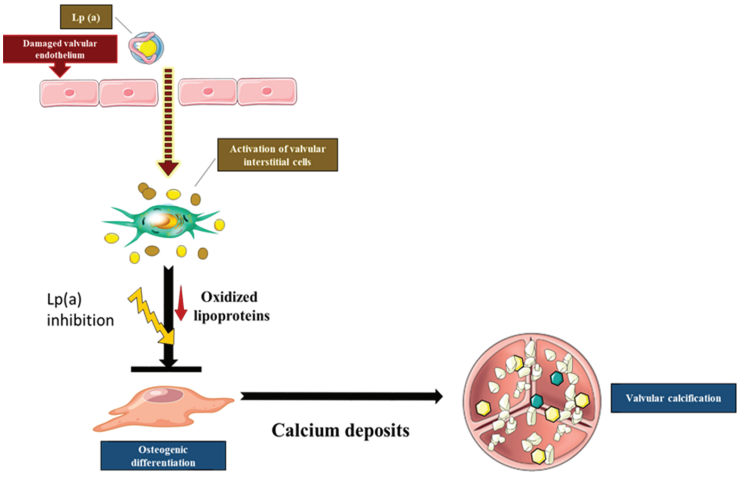

Figure 4. The effect of Lp(a) on aortic valve calcification. Lp(a) particles carry oxidized LDL, which stimulate aortic valve cells' calcification. Targeting Lp(a) might inhibit valve inflammation and, consequently, reduce valve cell calcification.

Proprotein convertase subtilisin/Kexin type 9 (PCSK9), an enzyme that is formed in the liver, has been described as a central player in cholesterol metabolism, particularly because PCSK9 stimulates the degradation of the LDL receptor and then leads to an increase in circulating LDL [75]. Inhibition of PCSK9 and its associated drastic reduction in circulating LDL suggests a promising treatment for patients with cardiovascular diseases [76,77]. In addition to lowering LDL, PCSK9 inhibitors were associated with a 15 to 30% reduction in Lp(a) plasma levels, which may benefit CAVD patients. Indeed, Langsted et al. [24] have shown, in 103,083 individuals from the Copenhagen General Population Study, that patients with loss-of-function mutation of PCSK9 (PCSK9 R46L) are associated with decreased

serum LDL and Lp(a) levels and a reduced risk of calcific aortic stenosis (CAVD). With this underlying substantial evidence, the PCSK9 inhibitors (deliberated as monoclonal antibodies: alirocumab and evolocumab) are currently being examined as pharmacological routes to delay the progression of aortic stenosis [78]. The exploratory investigations from the Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) randomized clinical trial support this approach. In this post-hoc analysis, patients randomized to evolocumab had a 50 % decrease in the incidence of CAVS over a median follow-up of 2.2 (1.8–2.5) years [79]. Furthermore, recent experimental studies [75,80] have shown that PCSK9 could be involved in the process leading to aortic valve calcification and that the in vitro inhibition of PCSK9 could decrease VIC calcification.

5.4. Statins' Off-Target Effects

Increased evidence points out several off-target effects of statins (Ward et al., 2019), including statin-associated muscle symptoms (SAMS), diabetes mellitus (DM), and effects on the central nervous system (Thompson et al., 2016) (Figure 5).

One of the main overlooked aspects in the context of statins' myotoxicity is calcium signaling. Studies showed that only lipophilic statins stimulate calcium release from the sarcoplasmic reticulum in rat and human skeletal muscle [81,82]. Acute applications of simvastatin on skeletal muscle fibers increased the cytosolic Ca^{2+} concentrations released from the sarcoplasmic reticulum [83]. Moreover, a previous study showed that simvastatin impaired ryanodine receptor 1 (RyR1) Ca^{2+} function, causing aberrant Ca^{2+} handling, which led ultimately to cell apoptosis [84].

The mevalonate pathway (Figure 2) produces ubiquinone or coenzyme Q10, which is an important player in the mitochondrial electron transport chain (ETC). Studies by Bouitbir and others showed clearly that statins impair mitochondrial function at different levels (Figure 5) [85–88]. For instance, simvastatin inhibits complex I activity, as observed in rat and human skeletal muscles treated with simvastatin [82]. The inhibition of mitochondrial function was only observed with lipophilic statins and was not rescued by the addition of cholesterol intermediate mevalonate, which would indicate that statins directly impair mitochondrial function, independently of the inhibition of HMG-CoA reductase [88]. Furthermore, patients treated with statins and experiencing muscle-related side effects presented impaired mitochondrial function, lower mitochondrial content, and increased mitochondrial reactive oxygen (ROS) production in deltoid biopsies [86]. However, the same study showed that statins increased mitochondrial function in heart biopsies of the same patients [86]. These findings showed clearly that oxidative muscles such as the heart are more resistant to statins than glycolytic muscles, most likely due to the higher mitochondrial content and higher anti-oxidative capacities (Figure 5).

Akt represents an important kinase governing the homeostasis between cell growth, survival, and metabolism. Several in vitro studies documented an impaired Akt function and its downstream signaling pathway, which lead to cell apoptosis and protein degradation [85,89]. Moreover, it was shown that decreased Akt phosphorylation and impaired mitochondria respiration were responsible for simvastatin-induced myopathy in C2C12 myo-tubes [90].

Recently, new-onset diabetes mellitus has been reported as a new adverse event in patients treated with statins [91]. The incidence of diabetes associated with statin therapy is estimated at up to 30% [92]. The JUPITER trial reported a significant increase in type 2 diabetes in patients treated with rosuvastatin [91]. Moreover, decreased insulin sensitivity and hyperglycemia in hypercholesteremic patients have been observed with atorvastatin [21]. However, the impairment of insulin sensitivity was observed only with lipophilic statins [21]. The impaired insulin signaling and the disturbed GLUT4 synthesis or translocation seem to establish the link between statins and insulin resistance [93]. Moreover, impaired translocation of the GLUT4 vesicles to the cell membrane was observed when cells were exposed to atorvastatin [94]. Statins impair the isoprenylation of several proteins and GTPases by inhibiting cholesterol biosynthesis. Interestingly, this effect was

not observed with pravastatin, highlighting a class effect. Because of these various off-target effects and the complex physiopathology of aortic stenosis, statins did not show any beneficial effect on stopping the progression of aortic stenosis.

Finally, the concept of a lipid-lowering therapeutic regimen in CAVS is reassuring. The heterogeneity of lipid species in the aortic valve might explain the failure of statins to reduce the progression of aortic stenosis. However, the clinical trials using antisense oligonucleotides to inhibit the expression of Lp(a) (Figure 4) or the use of monoclonal antibodies to inhibit the expression of PCSK9 still support the implication of lipids in Calcified Aortic Valve Disease. The use of lipidomic studies to explore the circulating and valvular lipid species in patients with CAVS might provide molecular cues for more efficient and compelling therapeutic targets to reduce the progression of aortic stenosis.

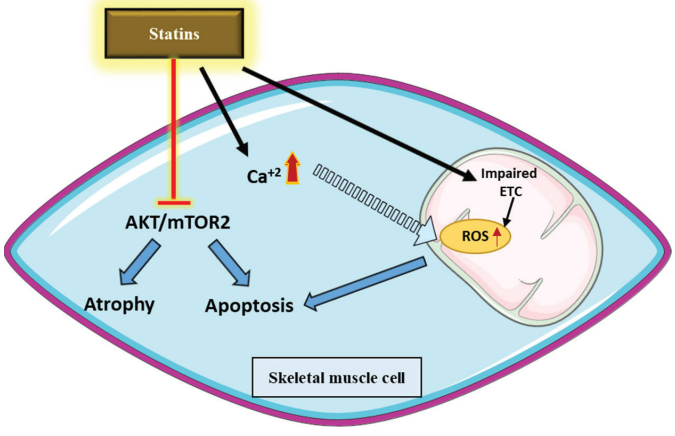

Figure 5. Off-target effects of statins. Statins act at different levels in skeletal muscle cells. First, they provoke the increased release of calcium from the sarcoplasmic reticulum to the cytoplasm and perturb the contractility of muscle fibers. In parallel, statins impair mitochondrial function, leading to the accumulation of ROS and to the activation of apoptosis. Finally, statins impair the function of Akt due to the impaired insulin signaling pathway and due to the impaired function of mTORC2. As a result, statins induce increased protein degradation and impaired protein synthesis, promoting skeletal muscle atrophy [95].

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Brief Report

The Effect of Celecoxib on the Progression of Calcific Aortic Valve Disease—Protective or Pathogenic?

Zachary Vinton [1,*], Kevin Wolfe [1], Jensen Fisher [2] and Amanda Brooks [3]

[1] College of Osteopathic Medicine, Rocky Vista University, Parker, CO 80112, USA
[2] Department of Library Services, Rocky Vista University, Parker, CO 80112, USA
[3] Office of Research and Scholarly Activity, Rocky Vista University, Parker, CO 80112, USA
* Correspondence: zachary.vinton@rvu.edu; Tel.: +1-(720)-940-5734

Abstract: Calcific aortic valve disease (CAVD) is a debilitating condition for which there are limited therapeutic options aside from valve replacement. As such, it is crucial to explore alternative management strategies for CAVD. Non-steroidal anti-inflammatory drugs (NSAIDs), particularly celecoxib, have been the subject of debate in the literature regarding their potential impact on CAVD. We conducted an in-depth analysis of five studies exploring the effect of celecoxib on CAVD and found discrepancies in both methods and results. Our findings suggest that celecoxib may impact the development of this disease via multiple mechanisms, each of which may have different effects on its pathogenesis. We also discovered limited clinical research examining the connection between celecoxib use and CAVD in medical patients. As such, further studies are needed to clarify the role of celecoxib and other NSAIDs in CAVD progression in order to inform future treatment options and clarify their impact on the disease.

Keywords: celecoxib; NSAIDs; COX-2; calcific aortic valve disease; aortic stenosis

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1. Introduction

Calcific aortic valve disease (CAVD) is accompanied by mineralization of bicuspid (half of all removed calcified valves are bicuspid) [1] or tricuspid aortic valve leaflets, leading to a progressive decline in function of the aortic valve via both decreased valvular area and increased valvular narrowing, resulting in a reduced blood flow through the leaflets [2]. Between 1990 and 2019, the global incidence of CAVD has increased by a factor of 3.51 (589,000). The prevalence has increased by 4.43 (9,404,000) and attributable deaths have increased by 1.38 (126,000), making it the most common valvular disorder and a significant cause of morbidity and mortality worldwide [3]. Due to systemic ramifications such as sudden death (in severe aortic stenosis), heart failure, pulmonary hypertension, infective endocarditis (particularly in patients with bicuspid valve calcification), bleeding, systemic emboli, and strokes [4], it is the most common indication for surgical valve replacement [5]. In fact, the only treatment modality currently available is surgery, which emphasizes the importance of uncovering new interventions as well as further illuminating the disease process behind CAVD.

2. Pathogenesis of CAVD

Previous hypotheses of the pathogenesis of CAVD included passive calcification and normal degeneration of the aortic valve; however, it has been found to be more complicated. Current understanding of the disease process includes chronic inflammation, lipoprotein deposition, and active leaflet calcification [6] contributing to progressive calcification. As shown in Figure 1, valvular interstitial cells (VIC) are the predominant aortic valvular cells and under normal circumstances are thought to reinforce valvular structure; however, they are suspected to be one of the disease drivers in CAVD, as they acquire pro-calcific

characteristics due to pathological stimuli such as lipoprotein accumulation, endothelial damage, inflammatory mediators, reactive oxygen species (ROS), increased calcium and phosphate levels, and cyclic stretch [4,6].

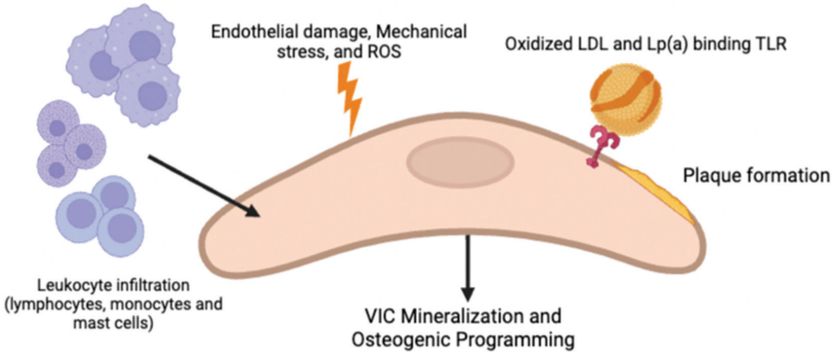

Figure 1. Pathogenesis of CAVD involves lesions from endothelial damage, mechanical stress, and ROS. This allows for oxidized LDL and Lp(a) infiltration, plaque formation, and subsequent leukocytic infiltration of VICs. This leads to progressive mineralization and osteogenic programming of VICs [4,6].

3. Inflammation

Similar to atherosclerosis, repeated endothelial damage is thought to be responsible for triggering the development of CAVD due to the loss of valvular homeostasis via reduced shear stress and increased mechanical stress. Subsequently, vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1), which are hallmarks of early stages of inflammation, are upregulated [1,7]. These cell signaling cascades lead to inflammatory cells (macrophages, T-Lymphocytes, and mast cells) being recruited into leaflets and the infiltration of lipoproteins (LDL and Lp(a)), forming subendothelial plaque-like lesions resulting from LDL oxidation due to the release of ROS from the inflammatory cells [1]. The binding of oxidized lipid species to TLRs on the VICs, as well as the activation of the NF-KB pathway via TNF (secreted by immune infiltrates such as macrophages and monocytes) and binding to TNFR1, are both thought to promote VIC mineralization and osteogenic programming [2]; however, how the pro-calcific VIC causes ECM mineralization is still not fully understood. In addition to osteogenesis, it is also postulated that apoptosis of VICs via ROS, cytokines, and purinergic signaling may lead to dystrophic calcification containing calcium and phosphorous crystals in CAVD [2]. Neovascularization often accompanies inflammation. Although the mechanism of its involvement in CAVD is not entirely clear, it is postulated that it is involved in recruiting both inflammatory cells and osteoprogenitor cells.

4. NSAIDs

The COX-1 and COX-2 pathways are responsible for converting arachidonic acid to products that mediate pain and inflammation, as shown in Figure 2 [8]. COX-1 produces thromboxane A2, and both COX-1 and COX-2 are responsible for the production of prostaglandins [9]. The COX-2 pathway, specifically, produces prostaglandins during inflammation [9]. COX inhibitors are a class of non-steroidal anti-inflammatory drugs (NSAIDs) that are used to block the COX-1 and COX-2 pathways to reduce pain, inflammation, and fever [8]. However, some COX inhibitors, such as COX-2 inhibitors (the majority of which have been discontinued from use) and aspirin, are associated with cardiovascular side effects [10]. It is still unknown whether COX inhibitors might play a role in the development of CAVD; however, studies have attempted to identify whether there is a connection due to the role these drugs play in inflammation and cardiovascular risk [10].

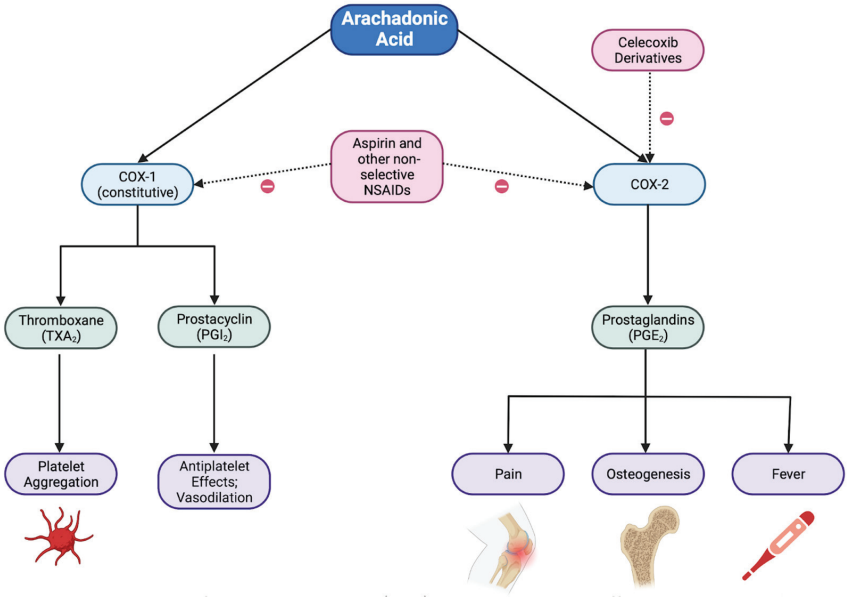

Figure 2. A summary of the cyclooxygenase (COX) pathways and their effects. Celecoxib and its derivatives selectively block the COX-2 pathway, inhibiting pain, inflammation, and fever without impacting platelet aggregation [7,8].

5. Mineralization

It is suspected that the cytokine IL-6, a central regulator in chronic and other immune-mediated responses plays a role in CAVD through its involvement in increasing the expression of NF-KB.2. IL-6 increased in human calcified stenotic valves, likely due to the expression of RANKL (receptor activator of an NF-KB ligand), which thereby activates RANK. RANKL causes VICs to increase the production of the extracellular matrix (ECM) [11]. Nucleation of calcium and phosphorus can begin on this secreted ECM. IL-6 also promotes mineralization through the BMP2 pathway [2]. Interestingly, Weiss et al. demonstrated that Osteoprotegerin administration, which is a decoy of RANKL, attenuated calcification of the aortic valve in mice and preserved valvular function [12]. The role of various proteins present in the ECM, such as proteoglycans and periostin, are thought to be involved in the remodeling of the aortic valve during aortic stenosis (AS), but this is not yet fully understood. For instance, osteopontin and bone sialoprotein are drastically upregulated at sites of calcification and help attach osteoblasts to bone matrix [13].

Other cytokines that may be involved are IL-1B and IL-1, which increase the expression of matrix metalloproteinases (MMPs). These enzymes degrade ECM, exacerbate stenosis, and activate the NF-KB pathway, leading to an increase in IL-6, IL-8, and MCP-1. IL-37, which attenuates bone morphogenic protein (BMP2) and alkaline phosphatase, both of which inhibit osteogenesis, is in the same family as IL-1B. In patients with CAVD, levels of IL-37 are low, leading to BMP2 promoting the thickening of the aortic valve [1]. Beyond the BMP pathways, both the angiotensin-converting enzyme (ACE) and chymase increased in CAVD. Chymase (via mast cells) and ACE both convert angiotensin I into angiotensin II. Angiotensin II (with a type AT1 receptor found in CAVD) [13] correlates with TNF and IL-6 expression and is pro-fibrotic [14], making it an important aspect in the pathogenesis of CAVD. In hypercholesterolemic rabbit models, it was found that angiotensin receptor-1 blockers (ARBs) were capable of preserving the endothelial integrity of the aortic valve while disrupting transdifferentiation into osteoblasts and/or myofibroblasts [15].

Additional factors thought to contribute to CAVD are genetic predispositions. Bicuspid valves, which are susceptible to calcification, are associated with NOTCH1 mutations.

Normally, NOTCH1 in VICs helps to prevent the expression of BMP2 and RUNX2, which are osteogenic factors, meaning that some patients may be genetically susceptible to developing CAVD. Moreover, the WNT pathways in patients with CAVD are overexpressed, which may also lead to calcification. The above factors contribute to the fibrosis and calcification of the aortic valve, ultimately leading to sclerosis and the necessity for surgical valve replacement. More research is necessary to illuminate the complexities behind the disease processes of CAVD.

6. Treatment Options

Currently, there are no treatments available for calcific aortic valve disease aside from surgical aortic valve replacement (SAVR) and transcatheter aortic valve implantation (TAVI/TAVR) (for patients with increased operative risk) [16]. This is problematic because of the risk for complications, including endocarditis and thrombosis, along with a limited valve lifespan, often leads to reoperation [4].

Based on the ACC/AHA Guideline for the Management of Patients with Valvular Heart Disease, an intervention for calcific aortic stenosis is only indicated if (1) CAVD is severe, (2) the patient has a life expectancy greater than one year with surgery, and (3) the intervention is likely to improve the patient's quality of life [17]. This is evaluated with a multidisciplinary heart valve team involving a cardiologist with expertise in structural valve intervention and a cardiothoracic surgeon. Indications for SAVR over TAVI include another indication for cardiac surgery (CABG or mitral valve surgery), patient age under 75, characteristics indicating a mechanical valve replacement (can only be placed surgically), or anatomic features increasing the risk of TAVI complications, such as adverse aortic root or a severely calcified bicuspid valve. If SAVR is not indicated, the transfemoral TAVI is a choice with a robustly lower hazard ratio and mortality. Notably, mortality was not reduced with transthoracic TAVI in comparison with SAVR and transfemoral TAVI. Indications for TAVI over SAVR include a patient aged 75 or higher, high feasibility of transfemoral TAVI, risk factors for SAVR (frailty or cirrhosis), and the female sex (lower mortality under TAVI compared to women w/SAVR). Risks for SAVR are evaluated using the STS risk estimate, frailty, major organ system dysfunction, and procedure-specific impediments. Patients are at intermediate risk, which is classified by an STS of 4–8%, when at least one indicator of frailty is present.

There are currently no drugs available for the treatment or prevention of aortic stenosis. Celecoxib, a selective COX-2 inhibitor, has been investigated as a potential solution to this gap in pharmacotherapeutic interventions [18]. There are two mechanisms by which the drug is proposed to prevent the progression of calcification of the aortic valve; however, at the time of writing this review, there has been limited research into this topic [18,19].

7. Materials and Methods

A comprehensive review of academic publications was performed to answer the following question: is there a connection between the use of celecoxib and the development of calcific aortic valve disease? An assessment of our current knowledge of this topic was accomplished by conducting a broad search of the literature, selecting relevant articles, and synthesizing the findings from each study in order to develop a uniform picture of our current understanding. The literature search was conducted in PubMed, Cochrane, Google Scholar, and ClinicalTrials.gov using the keywords NSAIDs, COX-2, calcific aortic valve disease, aortic stenosis, and celecoxib. Inclusion criteria consisted of studies that examined the possible connection between either the COX-2 pathway or celecoxib and the development of aortic valve calcification (AVC). Studies in both humans and animals that utilized quantitative data were accepted. Due to the focus of our review being on a topic that has not been studied robustly, further inclusion criteria were not incorporated. For the purpose of answering this question with up-to-date statistical information, exclusion criteria included studies that were published more than ten years ago and studies that utilized qualitative methodology.

8. Results

Due to the role of the COX pathways in inflammation and the known connection between NSAIDs and cardiovascular events [20], there has been an investigation as to whether the COX-2 pathway has a role in calcific aortic valve disease (CAVD). The investigation of this report identified five relevant studies, shown in Table 1, to help address this question and found that there is no current consensus in the literature regarding the connection between the two. Some studies suggest that upregulation of the COX-2 pathway could potentially be disease-driving in AVC [18]. However, others have suggested the opposite; that COX-2 has a protective effect against it. Therefore, COX-2 inhibitors could play a role in worsening the development of AVC and subsequent aortic stenosis [21]. Other studies have suggested that there may not be an association between COX-2 inhibitor use and AVC at all [22].

Table 1. A summary of the major findings of recent primary studies that investigated the potential association between celecoxib derivatives and the development of aortic valve calcification [18,19,21–23].

Study	Proposed Effect of COX-2 on CAVD Development	Proposed Effect of Celecoxib Derivatives on CAVD Development	Proposed Pathomechanism	Study Type
Delaney et al. [22]	No association	No association	–	Human retrospective study
Wirrig et al. [18]	COX-2 expression is increased in calcified aortic valves	Reduced development of calcification	COX-2 expression leads to increased osteogenic activity	Animal and human study-in vivo and in vitro
Vieceli Dalla Sega et al. [21]	COX-2 expression is decreased in calcified aortic valves	Increased development of calcification	COX-2 inhibition induces trans-differentation of AVIC's into myofibroblasts and increased expression of TGF-β	Human ex vivo study
Bowler et al. [19]	Not investigated	Celecoxib leads to increased calcification; DMC leads to decreased calcification	Myofibroblast induction increases calcification; CDH11 blockade decreases calcification	Human in vitro and retrospective study
Vaidya et al. [23]	Not investigated	Increased development of calcification in the presence of glucocorticoids	COX-2 inhibitors have an unknown glucocorticoid-dependent effect	Animal ex vivo study

One study found that the COX-2 pathway had increased expression in human calcified aortic valves [18]. That finding alone, however, does not necessarily mean that COX-2 drives calcification. More clarification is needed to determine whether COX-2 upregulation is driving calcific disease, or whether it means that upregulation is a protective response to another disease process causing the calcification. However, inhibition of the COX-2 pathway with celecoxib also reduced the induction of calcification in a mouse model, supporting the idea of a cause-and-effect relationship in which COX-2 activity leads to AVC. At a glance, this fits well considering COX-2 has a known role in bone healing [24], and these findings would suggest that celecoxib or other NSAIDs could potentially serve as therapeutics for AVC prevention.

However, another in vitro study performed on human aortic valve leaflets sampled from patients with aortic stenosis directly contradicts these findings [21]. In this study, it was found that the COX-2 pathway actually had decreased expression in calcified valves.

The addition of a COX-2 inhibitor, celecoxib, to these samples also induced further calcification. This would lend credence to the idea that the COX-2 inflammatory pathway has a protective effect, and its downregulation allows for calcification and subsequent stenosis to occur. As a result, it could be assumed that celecoxib and other COX inhibitors are risk factors for the development of aortic stenosis and would be contraindicated in patients at risk for it. As the primary cause of aortic stenosis is age-related calcification, this could be a major contraindication to a class of drugs already not widely used due to associations with other cardiovascular events (although celecoxib is proposed to be the safest in this regard) [25]. These findings are in direct opposition to those of Wirrig et al. (2015), although this difference was suggested to potentially be due to differences in the methods of measuring [18,21]. At the very least, however, the contradictory findings suggest that more investigation is needed to clarify this association.

There were also different proposed mechanisms regarding how celecoxib could affect the development of CAVD, which may help explain the contradictory findings. Celecoxib is proposed to have another non-COX-2-associated effect that could potentially be protective against AVC, through a CDH11 blockade [19]. The CDH11 transmembrane protein has been found to have increased expression in calcified aortic valves, serving as a potential therapeutic target [26]. Celecoxib and its derivatives have been shown to have a high binding affinity for this protein. Both celecoxib and dimethyl celecoxib (DMC), a celecoxib derivative with action against CDH11 but no inhibitory effects on COX-2, were investigated as potential therapeutics for the treatment of aortic stenosis. However, in one in vitro study, celecoxib was shown to actually be associated with increased calcification, further supporting the idea that COX-2 inhibition may be pathogenic, while DMC had the expected protective effect against calcification [19]. Vieceli Dalla Sega et al. (2020) agrees with these findings with regard to celecoxib, which again are in direct contradiction to Wirrig et al. (2015) [18,21].

More recently, another study performed a number of experiments with different conditions and variable findings that may explain the controversy as to whether the COX-2 inhibitors celecoxib and DMC are protective or pathogenic in the development of AVC [23]. Like previous studies, the authors replicated the potential increased risk for AVC in an in vitro environment using explanted porcine aortic valve leaflets in osteogenic media. This finding was observed with both celecoxib and DMC, suggesting that this potential pathogenic effect is not characteristic of COX-2 inhibition. However, this effect was not observed in studies that were performed without dexamethasone, suggesting that the pathogenic effect of celecoxib and DMC may be due to yet another non-COX-2-associated effect that is dependent on the presence of glucocorticoids. With dexamethasone removed from the osteogenic media, the effect was reversed as expected. Furthermore, the authors found that co-treatment with a MEK1/2 inhibitor rescued this pathogenic effect, suggesting an involvement of the MEK/ERK pathway in this glucocorticoid-dependent effect. The findings of this study, particularly the suggestion of another potential mechanism of action of celecoxib, may explain the previous controversy as to whether COX-2 inhibition is protective or pathogenic in the development of AVC if the reason for the contradictory findings in previous studies was the presence of glucocorticoids in in vitro media that were used.

These findings suggest both an explanation for the pathogenic effect of celecoxib and its derivatives, as well as the potential for therapeutic prevention of AVC, if these non-COX-2 effects can be further studied and understood. If a glucocorticoid-dependent effect causes the administration of celecoxib and its derivatives to drive calcification of the aortic valve, then it would suggest the need for studies of whether this effect is present in in vivo conditions when the drug is administered. It also suggests that if this pathway could be eliminated, such as with an MEK 1/2 inhibitor, celecoxib and DMC may still serve as potential therapeutics through the blockage of either COX-2 or CDH11. This highlights the need for further studies and suggests that celecoxib and its derivatives could either be potential therapeutics or disease-driving agents in CAVD and consequent aortic stenosis.

The impact of celecoxib and its derivatives on the development of AVC remains unclear with our current breadth of knowledge. However, there is evidence to suggest that celecoxib can affect three cellular pathways as shown in Figure 3, including the inhibition of COX2, CHD11 blockades, and potentially a third glucocorticoid-dependent effect. While there is debate as to whether COX2 inhibition can either promote or prevent AVC, it seems more certain that the CDH11 effect does help prevent its development. Conversely, it can be concluded that their effect in the presence of glucocorticoids may drive calcification. Due to these findings, the question of what celecoxib's role may be in the care setting remains unclear. However, these results provide a pathway forward for research to identify either new contraindications or therapeutic uses of celecoxib and its derivative drugs in the context of AVC.

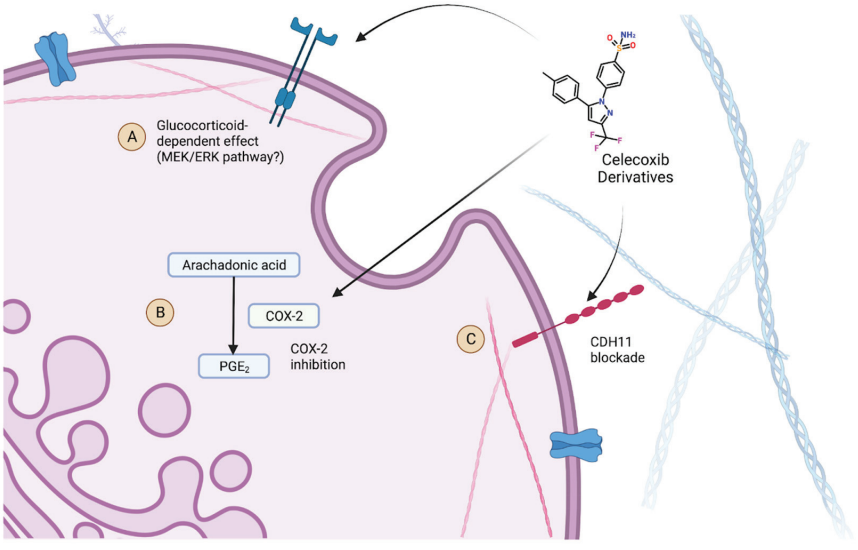

Figure 3. A summary of the pathways celecoxib and its derivatives that are proposed to affect (**A**) the glucocorticoid-dependent effect (possibly MEK/ERK), (**B**) the inhibition of COX-2, and (**C**) the blockage of the CDH11 transmembrane protein [9,19,23].

9. Discussion

Based on recent research findings, it is evident that the question of whether COX inhibition impacts the development of AVC, whether protective or pathogenic, requires further investigation. The only COX inhibitors that have had any recent investigation in this regard are celecoxib and its derivatives, and even that research is sparse and has conflicting results. In particular, there is a lack of studies on medical patients investigating this potential association between the COX pathways and AVC, with the exception of two retrospective clinical analyses with conflicting results [19,22]. There is a need for further retrospective studies of subjects taking COX inhibitors and simultaneously being monitored for the progression of aortic stenosis.

More research is also needed to clarify the role that celecoxib plays in AVC development in a manner that does not involve COX-2 inhibition. Since it is likely that there are other off-target pathways playing a role in celecoxib's effect on AVC, in order to determine the possibility of the COX pathways themselves having an impact on AVC, it may be necessary for other COX inhibitors to be studied as well in this regard. Despite the lack of knowledge about COX inhibition's role in the development of AVC, celecoxib clearly has a potential glucocorticoid-dependent effect that increases the risk of AVC development. Further investigation is needed as to whether this means the administration of celecoxib or

other COX inhibitors may lead to an increased risk for the development of aortic stenosis in an in vivo environment with exposure to serum glucocorticoids.

A potentially confounding variable influencing the results of studies with celecoxib is the CYP2C9*3 polymorphism that is found fairly frequently in Caucasian populations. CYP2C9 is a polymorphic enzyme involved in the metabolism of drugs such as NSAIDs, phenytoin, and (S)-warfarin, among others [27]. The genetic polymorphism CYP2C9*3 has been shown to lead to a statistically significant reduction in CYP2C9 activity of up to five- to ten-fold in homozygous carriers in in vitro studies [28]. Furthermore, there was more than a two-fold reduction in the oral clearance of celecoxib for homozygotes for CYP2C9*3 when compared to the wild type and heterozygotes [28], suggesting that patients with these mutations could be at risk for increased dose-related effects of celecoxib.

Notably, certain conclusions in this report are based on results from only one or two studies with varying methods, including studies that had findings directly contradictory to one another. These limitations, including the lack of available studies and inconsistency in study types, must be acknowledged. Although this report provides an overview of celecoxib and its implications in CAVD, it underscores the importance of further research to replicate these findings, given the significant clinical implications of potential pharmacotherapeutics for the prevention of CAVD.

10. Conclusions

As of the date of this review, aortic stenosis due to age-related wear and tear has no effective pharmacotherapy in widespread use. The progression of AVC to aortic stenosis necessitates surgical intervention; therefore, investigating potential therapeutics is highly important. Based on recent studies of celecoxib and its in vitro effects on the calcification of aortic valve leaflets, it is possible that it may have a mechanism of action, either due to a CDH11 blockade or through another mechanism, that could fulfill this need. As a result, more studies to identify the connection between celecoxib and AVC are needed for the dual purpose of better informing the current use of this drug and other COX-2 inhibitors, as well as identifying possibilities for potential pharmacotherapeutic intervention. Conversely, the possibility of celecoxib or other COX inhibitors' involvement in driving a pathogenic process leading to AVC and subsequent aortic stenosis should also be thoroughly investigated.

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Grosspeteranlage 5
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