

Mid-Term Outcomes of the Ozaki Procedure: A Single-Center Experience

ABSTRACT

Background: To assess the mid-term clinical and echocardiographic outcomes of the Ozaki (aortic valve neocuspidization (AVNeo)) procedure performed at a single center.

Methods: A total of 258 patients who underwent the AVNeo procedure between February 2019 and February 2025 were retrospectively analyzed. Demographic, operative, and echocardiographic data were evaluated. Patients were followed up with clinical examinations and transthoracic echocardiography at discharge, 3 months, 6 months, and annually thereafter.

Results: The mean follow-up duration was 36 ± 17.5 months. The average age was 56.4 ± 15.1 years, and 31.6% were female. The mean peak pressure gradient across the aortic valve was 17.0 (11.8–20.2) mm Hg immediately after surgery, then 14.0 (11.0–19.0) mm Hg at 1 year and 17.0 (12.0–23.0) mm Hg at 3 years. The Friedman analysis demonstrated a statistically significant change in peak pressure gradient over time ($\chi^2=68.103$, $P<.001$). Preoperatively, ejection fraction was 58.0 (52.0–64.0) %, increasing to 60.0 (56.0–62.0) % at 1 year and 61.0 (59.0–62.0) % at 3 years. Mild aortic regurgitation was seen in 2.7% of patients, and the reoperation rate was 0.38%. There were 3 in-hospital deaths (1.1%). Minimally invasive approaches (5 patients via a right anterior thoracotomy, 7 patients via an upper J sternotomy) were successfully performed in selected patients without needing to convert to full sternotomy.

Conclusion: The AVNeo procedure provides excellent mid-term outcomes with low complication and reoperation rates. Its compatibility with minimally invasive approaches and avoidance of anticoagulation make it a promising, durable alternative to conventional aortic valve replacement.

Keywords: Aortic valve neocuspidization, aortic valve reconstruction, autologous pericardium, minimally invasive cardiac surgery, Ozaki procedure

INTRODUCTION

Since Dr. Dwight Harken performed the first successful aortic valve replacement (AVR) in 1960, it has remained the standard treatment for aortic valve disease (AVD) and is now the most common heart valve surgery worldwide. Despite advances in prosthetic valve technology, an ideal prosthetic valve has not yet been developed. Mechanical heart valve prostheses require lifelong anticoagulation, which carries an estimated 2% annual risk of major bleeding after AVR.¹ On the other hand, bioprosthetic valves are mainly limited by structural valve degeneration, which often requires reoperation.² The growing popularity and standardization of repair techniques for atrioventricular valves, combined with the limitations of prosthetic valves, have increased interest in aortic valve repair for AVD.³ However, few centers perform this procedure, which is mostly used for aortic valve insufficiency, and few surgeons specialize in it.

The first documented aortic valve reconstruction using glutaraldehyde-treated autologous pericardium was reported by Duran et al.^{4,5} Building on this method, Ozaki et al standardized aortic valve reconstruction—now called the “Ozaki procedure”—by tailoring 3 different cusp sizes, usually based on the distance between the commissures, while accounting for the different hemodynamic stresses on each cusp.^{6,7} The Ozaki procedure has become a valuable treatment option for

ORIGINAL INVESTIGATION

Tuna Demirkıran¹ 

Yiğit Tokgöz¹ 

Veli Can Özdemir¹ 

Tayfun Özdemir¹ 

Emre Kubat² 

Gökhan Erol¹ 

Murat Kadan³ 

Serdar Fırtına⁴ 

Salim Yaşar⁴ 

Kubilay Karabacak¹ 

¹Department of Cardiovascular Surgery, Gülhane Training and Research Hospital, University of Health Sciences, Ankara, Türkiye

²Department of Cardiovascular Surgery, İskenderun Gelişim Hospital, Hatay, Türkiye

³Department of Cardiovascular Surgery, Memorial Ankara Hospital, Ankara, Türkiye

⁴Department of Cardiology, Gülhane Training and Research Hospital, University of Health Sciences, Ankara, Türkiye

Corresponding author:

Kubilay Karabacak

✉ kubilaykarabacak@yahoo.com

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various aortic valve conditions, including aortic stenosis (AS), aortic regurgitation (AR), and infective endocarditis, and its use is growing globally.⁸

The aortic valve neocuspidization (AVNeo) procedure was introduced at the center in February 2019. This study aims to evaluate the mid-term clinical outcomes of patients who underwent the AVNeo procedure at the institution.

METHODS

Study Design and Patient Population

The Institutional Ethics Committee approved the study protocol (Approval date: October 9, 2025, No: 2025/176). The first consecutive 258 patients who underwent the AVNeo procedure at the center between February 2019 and February 2025 were included in the study. Data were collected and analyzed retrospectively to evaluate the mid-term clinical outcomes of the procedure after receiving ethics committee approval. Throughout the study period, the institution routinely performed both conventional AVR and the AVNeo procedure. The AVNeo procedure was offered to patients with suitable valve anatomy who declined prosthetic valves, had a small aortic annulus with a risk of patient–prosthesis mismatch, or were deemed good candidates based on the heart team’s assessment. Patients requiring redo aortic valve surgery were excluded from the study. Additionally, the AVNeo procedure was avoided at the institution when usable autologous pericardium was unavailable (e.g., due to prior pericarditis or prior thoracic radiation). All patients provided informed consent before surgery. They underwent routine postoperative follow-up with clinical evaluations and transthoracic echocardiography at 3 months, 6 months, and annually afterward. The study was conducted in accordance with the Declaration of Helsinki.

Surgical Technique

The surgical procedure was performed through a median sternotomy in 244 patients. As the expertise in the AVNeo technique and minimally invasive cardiac surgery increased, alternative approaches in select cases with isolated aortic valve issues were used: 5 patients underwent surgery via a right anterior thoracotomy, and 7 patients were treated

through an upper J sternotomy. All cases employed standard cannulation techniques and routine myocardial protection with antegrade cold blood cardioplegia. Autologous pericardium was harvested and prepared to at least 7 × 8 cm. Proper sizing was emphasized as a key factor for procedural success. After excising and customizing the native aortic valve cusps, new pericardial cusps were implanted using the standardized AVNeo procedure (Figure 1).^{6,79} In patients with bicuspid or unicuspid morphology, tricuspidization was performed (Figure 2).^{9,25}

Statistics

Continuous variables were assessed for normal distribution using the Shapiro–Wilk test and visual inspection of Q–Q plots. Data that followed a normal distribution were reported as mean ± standard deviation, while non-normally distributed variables were presented as median [interquartile range (IQR)]. Categorical data were summarized with frequencies and percentages. Since the study included multiple surgical groups with repeated echocardiographic measurements across follow-up periods (preoperative, 1st month, 3–12 months, and beyond 12 months), a repeated-measures analysis was applied. As continuous echocardiographic variables were predominantly non-normally distributed, within-group changes over time were analyzed using the Friedman non-parametric 2-way ANOVA. Post-hoc pairwise comparisons between time points were performed using the Wilcoxon signed-rank test with Bonferroni correction (adjusted significance threshold: $\alpha = 0.05/6 = 0.0083$). Between-group comparisons at each time point were performed using the Kruskal–Wallis test. For significant Kruskal–Wallis results, post-hoc pairwise comparisons were conducted using the Mann–Whitney *U*-test with Bonferroni correction ($\alpha = 0.05/3 = 0.017$). All statistical analyses were conducted using IBM SPSS Statistics, version 26 (Armonk, NY, USA).

RESULTS

Preoperative Characteristics

The study cohort had a mean age of 56.4 ± 15.1 years (range 17–80), with females comprising 31.6% ($n = 79$). Detailed clinical and preoperative echocardiographic baseline characteristics are shown in Tables 1 and 2. Valve morphology was bicuspid in 17% ($n = 44$), unicuspid in 0.003% ($n = 1$), and tricuspid in the remaining 82.5% ($n = 213$). The median logistic EuroSCORE was 3.0 (1.0–4.0).

Operative Data

Concomitant surgical interventions included coronary artery bypass grafting (CABG) in 18 patients, mitral valve replacement (MVR) in 21, and ascending aortic surgery in 36 patients (comprising 19 Florida sleeve procedures and 17 supracoronary ascending aortic replacements). Additionally, 1 patient underwent combined MVR and ascending aortic surgery, 3 had combined MVR and CABG, one had CABG with ascending aortic surgery, and 1 had simultaneous CABG and carotid endarterectomy. The mean durations for cardiopulmonary bypass and aortic cross-clamp were 166.6 ± 47.8 minutes and 122.5 ± 30.9 minutes, respectively. For isolated AVNeo procedures, these times were shorter, averaging $132.6 \pm$

HIGHLIGHTS

- The Ozaki procedure showed excellent mid-term hemodynamic performance and valve durability in 258 patients.
- The technique demonstrated low reoperation rates (0.38%) and minimal postoperative complications, with no need for prosthetic conversion.
- The technique was feasible in complex combined cardiac surgeries and through minimally invasive approaches, including right anterior thoracotomy and upper J sternotomy, and was safely implemented.
- The Ozaki procedure maintained natural aortic annular dynamics, showed good hemodynamic performance even in patients with small aortic annuli, and eliminated the need for lifelong anticoagulation therapy.

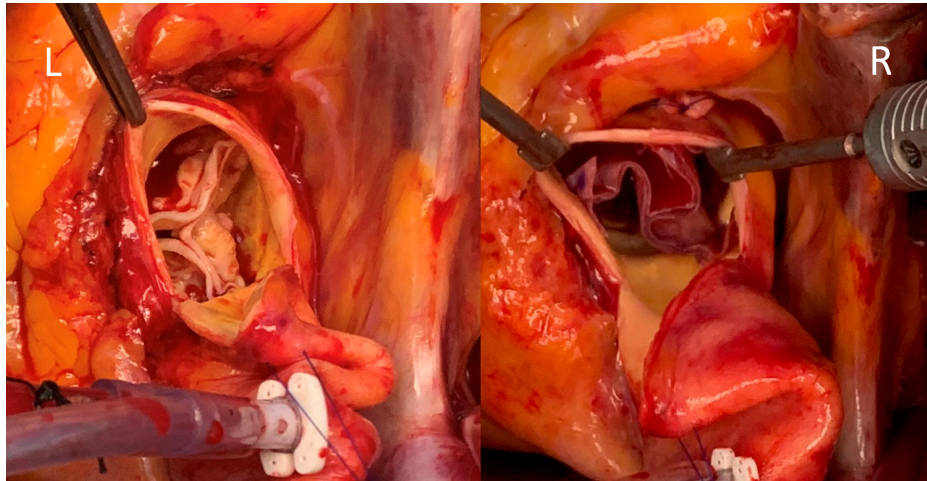


Figure 1. Intraoperative images before and after the AVNeo (Ozaki) aortic valve reconstruction. The image on the left displays the native aortic valve with calcified cusps before removal, while the image on the right shows the reconstructed valve with autologous pericardial leaflets after the procedure.

36.9 minutes for cardiopulmonary bypass and 101.7 ± 24.3 minutes for cross-clamping. None of the minimally invasive approaches required conversion to full median sternotomy. Intraoperative transesophageal echocardiography confirmed satisfactory hemodynamic function of all reconstructed aortic valves, with no cases needing conversion to prosthetic valve replacement.

Postoperative Outcomes

Hospitalization and ICU stay: The average length of stay in the intensive care unit was 1.2 ± 0.8 days, while the total hospital stay averaged 6.5 ± 2.1 days.

Echocardiographic findings: The mean follow-up period was 36 ± 17.5 months. PredischARGE transthoracic echocardiography showed a mean peak pressure gradient of 17.0 (11.8–20.2) mm Hg, with mild AR identified in 7 patients. At 1-year and 3-year follow-ups, mean peak gradients were

14.0 (11.0–19.0) mm Hg and 17.0 (12.0–23.0), respectively. The Friedman analysis showed a statistically significant change in peak pressure gradient over time ($\chi^2 = 68.103$, $P < .001$). Post hoc Wilcoxon analysis with a Bonferroni correction confirmed that the gradient decreased significantly from preoperative values at all postoperative time points (all $P < .001$), with no significant differences between postoperative time points (all $P > .0083$), indicating stable, sustained hemodynamic improvement. One patient who underwent an isolated AVNeo procedure required reoperation due to grade 2 AR at 2 months postoperatively. A significant improvement in left ventricular function was observed in patients after the Ozaki procedure. Preoperatively, ejection fraction was 58.0 (52.0–64.0)%, increasing to 60.0 (56.0–62.0)% at 1 year and 61.0 (59.0–62.0)% at 3 years. A Friedman test showed a statistically significant change over time ($\chi^2 = 30.314$, $P < .001$). Post hoc pairwise comparisons indicated that the

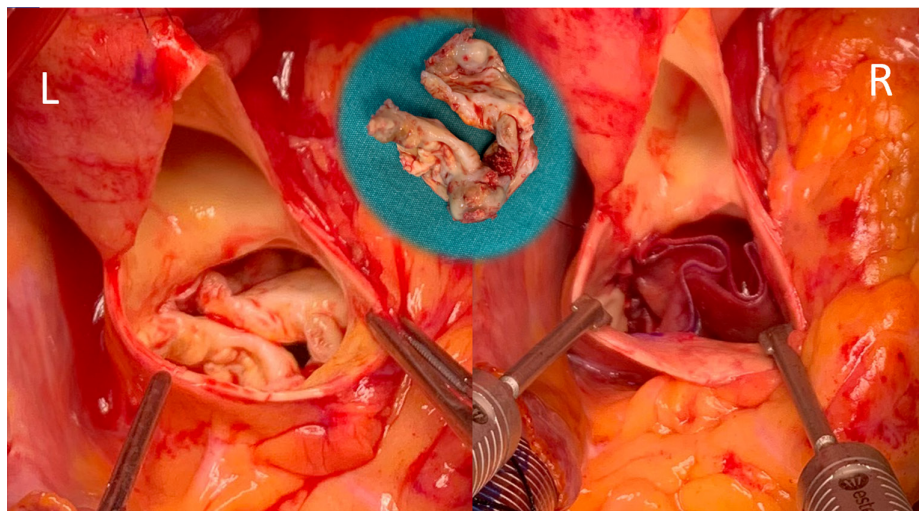


Figure 2. Intraoperative images showing the morphology of a bicuspid native aortic valve before removal (left), the excised native valve leaflets (center), and the reconstructed aortic valve with autologous pericardial cusps after completing the tricuspidization procedure (right).

Table 1. Preoperative Clinical Characteristics of the Patients

	Number of Patients	Percentage (%)
Diabetes mellitus	73	28.29
Hypertension	154	59.68
Hyperlipidemia	36	13.95
Coronary artery disease	59	22.86
Previous cerebrovascular accident	6	2.32
Glomerular filtration rate		
<30	6	2.32
30-60	18	6.97
>60	234	90.69

improvement became significant at 1 year and was maintained at 3 years ($P < .001$ for both comparisons vs. baseline). The median left ventricular end-diastolic diameter was 60.0 (57.0–62.0) mm preoperatively and decreased significantly to 52.0 (49.0–55.0) mm at 1 year, further declining to 49.0 (46.0–52.0) mm at 3-year follow-up. A Friedman test showed a statistically significant change over time ($\chi^2 = 52.751$, $P < .001$). Post hoc pairwise comparisons confirmed significant reductions at both 1 year and 3 years compared with baseline ($P < .001$), consistent with progressive and sustained ventricular reverse remodeling. Within-group temporal changes

Table 2. Preoperative Echocardiographic Characteristics of Patients

Parameters	Values
Ejection fraction, (IQR) (%)	58.0 (52.0–64.0)
≥55	190 (73.64)
45–54	29 (11.24)
36–44	24 (9.3)
≤35	15 (5.81)
Left atrium (mm)	42.2 ± 7.1
Left ventricular end-diastolic diameter, (IQR) (mm)	60.0 (57.0–62.0)
Aortic annulus (mm)	23.3 ± 2.6
Pathology (%)	
Aortic stenosis (AS)	127 (49.22)
Aortic regurgitation (AR)	88 (34.1)
Mixed lesion (AS + AR)	43 (16.66)

in echocardiographic parameters for the surgical subgroups (isolated AVNeo, AVNeo + CABG, AVNeo + MVR, ascending aortic procedures) and between-group comparisons at corresponding follow-up time points are presented in Table 3.

Anticoagulation strategy: Routine anticoagulation was not given after isolated AVNeo procedures. Patients without other reasons for anticoagulation received 100 mg of aspirin

Table 3. Echocardiographic Parameters in the Preoperative and Postoperative Follow-up Periods According to Concomitant Surgical Procedure Groups

Variables	Time	Group 1 Isolated AVNeo	Group 2 AVNeo + CABG	Group 3 AVNeo + MVR	Group 4 AVNeo + AAS	P (Between Groups) Kruskal–Wallis	P (Over time) Friedman G1 / G2 / G3 / G4
Ejection fraction (%)	Preoperative	60.0 (60.0–65.0)	60.0 (60.0–65.0)	60.0 (50.0–60.0)	60.0 (60.0–65.0)	<.001*	G1: <.001*** G2: .024* G3: .201 NS G4: .002**
	Predischarge	60.0 (60.0–60.0)	60.0 (55.0–60.0)	55.0 (50.0–60.0)	60.0 (55.0–60.0)	.082	
	1 st year	60.0 (60.0–60.0)	60.0 (52.5–60.0)	58.0 (50.0–60.0)	60.0 (60.0–65.0)	<.001*	
	3 rd year	60.0 (60.0–61.2)	60.0 (60.0–65.0)	60.0 (55.0–60.0)	60.0 (60.0–60.0)	.032*	
Maximum gradient (mm Hg)	Preoperative (Aortic stenosis)	73.0 (65.8–96.0)	74.0 (54.0–90.0)	75.0 (40.8–83.0)	65.5 (26.0–76.2)	.043*	G1: <.001*** G2: .004** G3: .710 NS G4: .005**
	Predischarge	16.5 (12.0–20.0)	17.0 (14.0–21.0)	10.5 (10.0–21.0)	17.0 (12.0–20.0)	.410	
	1 st year	16.5 (12.0–23.0)	13.0 (11.2–18.5)	13.0 (11.0–14.0)	12.0 (10.0–18.0)	.127	
	3 rd year	17.0 (12.0–21.0)	19.5 (16.5–23.2)	22.0 (13.0–24.0)	12.0 (9.0–14.8)	.039*	
Mean gradient (mm Hg)	Preoperative (Aortic stenosis)	46.0 (41.0–58.0)	43.0 (37.2–60.0)	48.0 (36.8–55.8)	41.0 (30.0–52.0)	.053	G1: <.001*** G2: .670 NS G3: .740 NS G4: .002**
	Predischarge	11.0 (9.0–15.8)	9.0 (5.5–11.8)	5.0 (3.0–12.0)	8.0 (7.5–10.0)	.122	
	1 st year	10.0 (8.2–14.5)	8.5 (6.5–10.2)	7.0 (4.0–8.0)	8.0 (5.0–11.0)	.074	
	3 rd year	8.5 (5.0–13.0)	11.0 (10.2–14.8)	10.0 (6.0–14.0)	4.0 (4.0–10.5)	.263	

Data are presented as median (IQR). P (between groups): Kruskal–Wallis test. P (Over time): For each group, a separate Friedman non-parametric 2-way ANOVA (G1–G4) was performed. Post-hoc comparisons were conducted using the Wilcoxon signed-rank test with Bonferroni correction ($\alpha = 0.0083$).

AAS, ascending aortic surgery; CABG, coronary artery bypass graft; MVR, mitral valve replacement; NS, not significant.

* $P < .05$, ** $P < .01$, *** $P < .001$.

daily for 1 month after surgery. One patient had an ischemic stroke on postoperative day 7; no other thromboembolic events were reported.

Early postoperative complications: Eight patients (3.1%) required surgical re-exploration for bleeding in the early postoperative period, including 2 who underwent isolated AVNeo procedures. There were 3 in-hospital deaths: 1 from low cardiac output syndrome on postoperative day 1 (combined AVNeo and bioprosthetic MVR operation), 1 due to mesenteric ischemia on day 3 (isolated AVNeo procedure), and 1 from ischemic stroke on day 7 (isolated AVNeo procedure). A permanent pacemaker was implanted in 1 patient with severe annular calcification.

DISCUSSION

This study reinforces the AVNeo procedure as a safe, effective, and reproducible option for aortic valve reconstruction, showing excellent mid-term clinical and echocardiographic outcomes. The procedure's adaptability to minimally invasive approaches and complex concomitant surgeries further expands its applicability. The results are consistent with and add to the growing evidence supporting the AVNeo technique as a valuable alternative to traditional prosthetic valve replacement.

The cohort included the first consecutive 258 patients who underwent the AVNeo procedure at the center over 6 years. The average cardiopulmonary bypass and cross-clamp times are consistent with previous reports, despite a significant number of patients undergoing additional procedures such as CABG, MVR, and ascending aortic surgery.⁷⁸ This indicates that the AVNeo technique can be safely incorporated into complex cardiac surgeries without substantially increasing operative time.

While most procedures were performed via median sternotomy, the use of minimally invasive approaches—including right anterior thoracotomy and upper J sternotomy—in selected cases without converting to full sternotomy or prosthetic valve replacement highlights the growing versatility and safety of the AVNeo procedure. These findings complement recent literature showing the feasibility of minimally invasive AVNeo surgery, which may reduce surgical trauma and promote faster recovery.^{10,11} To the best of the knowledge, this is the first report in the literature describing the performance of the Ozaki procedure through a right anterior thoracotomy using a direct vision approach.

Hemodynamic performance was excellent, with mean peak pressure gradients remaining low and stable over a mean follow-up of 36 months. Only a small subset of patients exhibited mild AR, and the reoperation rate was low (0.38%). Additionally, there was a gradual decrease in left ventricular dimensions during the first postoperative year, which may reflect favorable reverse remodeling following valve reconstruction. These outcomes align with prior studies showing durable valve function and favorable hemodynamics of glutaraldehyde-treated autologous pericardial cusps.^{8,12} The

low rate of significant regurgitation and reoperation indicates good mid-term valve function.

Although aortic valve repair avoids the disadvantages of prosthetic valves, it is still performed in a limited set of conditions, mainly aortic insufficiency, and only by a few surgeons.¹³ Bioprosthetic valves, which eliminate the need for lifelong anticoagulation, have durability limitations, whereas mechanical valves require lifelong anticoagulation with associated risks. Additionally, neither type of prosthesis can achieve hemodynamics as favorable as those of the native aortic valve. In a healthy aortic valve, opening begins with systolic dilation of the annulus, creating a maximal effective orifice area that reduces stress on the aortic cusps. As a result, even if the healthy native aortic valve has a small annulus, it maintains a low gradient. Conversely, prosthetic valves restrict annular motion throughout the cardiac cycle because they are fixed with a rigid ring. The most significant advantage of the AVNeo procedure is the preservation of this dynamic function.^{14,15} Additionally, full systolic opening of each cusp in a reconstructed valve helps achieve the maximum effective orifice area. It also maintains coordination between the left ventricle and the aortic root during the cardiac cycle, leading to excellent hemodynamic results, as shown in the study.

The incidence of calcific AS has increased approximately fourfold over the past 3 decades, and aortic valve stenosis is now the most common valvular pathology in developed countries.^{16,17} The increasing prevalence of AS has led to more patients with small aortic annuli, requiring advanced surgical techniques such as annular enlargement or complete root replacement to prevent patient-prosthesis mismatch. These procedures pose risks, including perioperative mortality, readmission, reoperation, and impacts on long-term survival.^{18,19} The AVNeo procedure eliminates these risks by maintaining native annular dynamics and offering better hemodynamic performance without requiring annular enlargement or root replacement, especially in older patients.

Durability in aortic valve repair largely depends on the effective height between the free edges of the cusps and their attachment points, which should be at least 8–9 mm. In the AVNeo procedure, the free edges of the cusps are raised to the level of the sinotubular junction, ensuring an effective height of at least 10 mm.²⁰ Also, glutaraldehyde-treated autologous pericardium has superior mechanical strength—4 times greater than non-calcified native valves and 10 times greater than calcified valves—and shows a low tendency for calcification.²¹ Additionally, using autologous tissue lowers the risk of infection by avoiding foreign materials. These factors together support the long-term durability and function of the reconstructed valve. Furthermore, autologous pericardium is easily accessible and cost-effective for all patients.

Valve-in-valve transcatheter aortic valve replacement (ViV TAVR) offers a less invasive option than redo surgery for high-risk patients with failing bioprosthetic valves, but it is

limited by patient-prosthesis mismatch, elevated residual gradients, and the risk of coronary obstruction.²² Although not part of this cohort, the AVNeo procedure's preservation of native annular dynamics may support future transcatheter interventions without the limitations seen in ViV TAVR, providing a clear advantage.²³

Our study also highlights low thromboembolic complication rates and the avoidance of routine anticoagulation in isolated AVNeo cases, providing quality-of-life benefits over mechanical prostheses, especially for younger patients.

The AVNeo technique is feasible and applicable to various aortic valve pathologies, including AS, AR, and infective endocarditis. It can also be adapted for bicuspid, unicuspid, and quadricuspid morphologies.^{9,24,25} Additionally, it is compatible with root reimplantation procedures for annuloaortic ectasia.¹² In this series, 36 patients underwent the AVNeo procedure along with ascending aortic surgery, including the Florida sleeve technique and supracoronary ascending aortic replacement. The previous study introduced a novel combination of the Florida sleeve with AVNeo reconstruction for patients with concomitant AVD and sinus of Valsalva aneurysm.²⁶ This novel approach has been used for the simultaneous treatment of sinus of Valsalva aneurysms with aortic valve abnormalities, providing a comprehensive surgical strategy that combines the benefits of both procedures.

This study presents a large series of 258 patients undergoing the AVNeo procedure at a single center. The sizable sample and systematic follow-up enhance the reliability and generalizability of the findings, providing one of the most comprehensive mid-term evaluations of this surgical technique to date. By including not only the standard median sternotomy but also minimally invasive approaches like right anterior thoracotomy and upper J sternotomy, the study demonstrates the versatility and adaptability of the AVNeo procedure in real-world clinical settings. Additionally, the inclusion of patients undergoing complex combined surgeries (e.g., CABG, MVR, ascending aortic surgery) highlights the procedure's feasibility and safety in complex cardiac interventions. This research adds to the growing global evidence supporting the AVNeo procedure as a viable alternative to traditional valve replacement.

CONCLUSION

The AVNeo procedure is a safe, effective, and feasible option for aortic valve reconstruction, especially advantageous for patients with small aortic roots or those seeking alternatives to prosthetic valves. Its compatibility with minimally invasive approaches and simultaneous surgeries broadens surgical choices, potentially enhancing recovery and minimizing trauma. The low thromboembolic risk and the avoidance of lifelong anticoagulation further increase its attractiveness, particularly for younger patients. These promising mid-term results justify further long-term and multicenter studies to determine durability and compare its effectiveness with traditional valve replacement methods.

Study Limitations

Although the study offers valuable mid-term clinical and echocardiographic outcomes with an average follow-up of about 36 months, longer-term data are needed. The results are based on a single-center cohort, which could limit how widely the findings apply. Differences in surgical skills, patient selection, and perioperative care across centers can affect outcomes. Multi-center studies would help confirm reproducibility and wider applicability. Without a randomized comparison to traditional prosthetic valve replacement or other surgical methods, it's hard to definitively determine superiority or equivalence. The study includes relatively few patients with unicuspid, bicuspid, and quadricuspid valve morphologies, as well as those undergoing minimally invasive approaches, which may restrict conclusions about these groups. Additionally, the influence of concomitant procedures on long-term valve function needs further investigation.

Ethics Committee Approval: This study was approved by Gülhane Training and Research Hospital Non-Interventional Research Ethics Committee (Approval date: October 9, 2025, No: 2025/176).

Informed Consent: Informed consent was obtained from all participants.

Peer-review: Externally peer-reviewed.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

AI-Assisted Technologies Statement: Artificial intelligence-assisted technologies were not used in the production of the submitted study.

Author Contributions: Concept – T.D., Y.T., T.Ö., K.K.; Design – T.D., T.Ö., G.E., K.K.; Supervision – K.K., M.K., S.F.; Resources – M.K., S.F., S.Y., K.K.; Materials – V.C.Ö., T.Ö., E.K., S.Y., K.K.; Data Collection and/or Processing – V.C.Ö., E.K., Y.T., G.E.; Analysis and/or Interpretation – T.D., S.Y., Y.T., V.C.Ö.; Literature Search – T.D., S.F., E.K., T.Ö., K.K.; Writing – T.D., K.K., G.E.; Critical Review – K.K., M.K., S.F.

Declaration of Interests: The authors have no conflicts of interest to declare.

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