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ORIGINAL RESEARCH

STRUCTURAL

Hydrodynamic Assessment of Explanted Degenerated Transcatheter Aortic Valves

Novel Insights Into Noncalcific and Calcific Mechanisms

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ABSTRACT

BACKGROUND The etiology of transcatheter aortic valve (TAV) degeneration is poorly understood, particularly non-calcific mechanisms.

OBJECTIVES The authors sought to investigate noncalcific and calcific mechanisms of TAV degeneration and evaluate their impact on leaflet function by bench testing, imaging, and histology.

METHODS TAV explants were obtained from the EXPLANT THV registry and clinical institutions. Hydrodynamic assessment was performed using a heart valve pulse duplicator system under physiological conditions. Micro-computed tomography, high-resolution photography, high speed video, and hematoxylin and eosin staining were used to evaluate the morphological appearance, leaflet kinematics, and calcium burden of TAVs.

RESULTS A total of 14 explants were evaluated: 10 self-expanding CoreValve/Evolut TAVs (Medtronic), 3 balloon-expandable SAPIEN 3 TAVs (Edwards Lifesciences), and 1 mechanically expandable Lotus TAV (Boston Scientific). The median patient age at explantation was 73.0 years (Q1-Q3: 64.5-80.0 years), with a time to explantation of 4 years 1 month (1 year 5 months to 4 years 11 months). Six TAV explants were found to have leaflet calcification (162.4 mm^3; 58.8-603.0 mm^3), and 8 had no calcification detectable by micro-computed tomography and histology. All samples had impaired leaflet kinematics. There was no significant difference in the hydrodynamic mean gradient between calcified (47.2 mm Hg; 26.6-74.1 mm Hg) and noncalcified (27.6 mm Hg; 15.2-36.7 mm Hg; $P = 0.28$) TAVs. Leaflet calcification had a weak but nonsignificant association with the hydrodynamic mean gradient ($r = 0.42$; $P = 0.14$).

CONCLUSIONS TAV function can be severely impacted by noncalcific and calcific mechanisms of tissue degeneration. Importantly, functional stenosis can occur in TAVs in the absence of obvious and significant calcification.

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Transcatheter aortic valve replacement (TAVR) is a treatment option for patients with severe calcific aortic stenosis (AS) irrespective of surgical risk.[1] As TAVR expands to younger patients, the optimal treatment options over a patient's lifetime are unknown. All bioprosthetic valves have the potential to fail, and transcatheter aortic valves (TAVs) may degenerate. Currently, there is good mid-term durability for TAVs, but the long-term durability and mechanisms of degeneration are poorly understood.[2,3] It is crucial to investigate the mechanisms of TAV degeneration, as they have an impact on treatment selection at the time of the index TAVR, and on the feasibility of repeat procedures in the event of TAV degeneration.

Bioprosthetic valve degeneration is frequently characterized by leaflet calcification. However, prior studies in surgical bioprosthetic aortic valves have shown that up to 25% of reoperations occur due to degeneration in valves that have minimal calcification.[4] Noncalcific mechanisms of bioprosthesis failure may include protein infiltration, oxidative stress, protein glycation, microstructural changes, and/or fibrosis. Failure of the intrinsic biomaterials of the bioprosthesis can manifest clinically as stenosis, regurgitation, or both.[5-8] Calcific and noncalcific mechanisms of degeneration in TAVs remain poorly understood. Additionally, these degenerative mechanisms that can manifest as clinical symptoms or abnormal echocardiographic parameters have an unknown impact on leaflet function and kinematics. In this study, we sought to better understand the noncalcific and calcific mechanisms of TAV degeneration and evaluate their impact on leaflet kinematics and TAV function.

ABBREVIATIONS AND ACRONYMS

AS = aortic stenosis

CT = computed tomography

EOA = effective orifice area

NSVD = nonstructural valve dysfunction

SVD = structural valve deterioration

TAV = transcatheter aortic valve

TAVR = transcatheter aortic valve replacement

METHODS

DATA SOURCE AND STUDY DESIGN. A total of 14 TAV explants were analyzed in this study: 10 supra-annular, self-expanding CoreValve/Evolut TAVs (Medtronic), 3 intra-annular, balloon-expandable SAPIEN 3 TAVs (Edwards Lifesciences), and 1 intra-annular, mechanically expandable Lotus TAV (Boston Scientific) (Table 1). Nine explants were obtained from the EXPLANT THV Registry based at St. Paul's Hospital and The Centre for Heart Lung Innovation Cardiovascular Tissue Registry, and approved by Providence Health Care Research Ethics Board. The EXPLANT THV Registry is an international multicenter registry of explanted TAVs coordinated by the Cardiovascular Translational Laboratory (Vancouver, BC, Canada). The remaining 5 explants were obtained from clinical institutions after obtaining local institutional review board approvals. Clinical data and patient characteristics were provided by each site when available.

Samples were only included for this series if they were intact and suitable for ex vivo hydrodynamic testing. TAV explants that were severely deformed or crushed at the time of explant were not included. For this reason, a total of 6 explants originally collected were excluded from this study. Biosafety protocols were followed for the collection and transfer of all samples. Samples were placed in buffered aldehyde fixative at sites almost immediately after explantation and stored at controlled room temperatures within the sample registries. All data were deidentified and transmitted securely. This study was performed under physiological ex vivo test conditions with no human or animal participants. Before hydrodynamic testing, samples were all transitioned to 0.2% buffered glutaraldehyde solution before being placed in a hydrodynamic test saline solution in order to provide significant washout before data collection.

All patients had TAV surgical explantation for clinical indications as deemed by their treating heart team. Reasons for explantation included: structural valve deterioration (SVD), nonstructural valve dysfunction (NSVD), endocarditis, and transplantation. Patients who required emergency surgical conversion immediately after TAVR or surgical intervention within the same hospitalization were excluded from the study. From the 14 TAV explants, 4 samples had been implanted as a second bioprosthesis after a valve-in-valve procedure (2 TAV-in-surgical aortic valves, 1 TAV-in-TAV, 1 not available).

HYDRODYNAMIC TESTING OF EXPLANTED TAVs. Ex vivo hydrodynamic measurements in this study were performed using a commercial heart valve pulse duplicator system (HDTi-6000, BDC Laboratories). Explanted TAVs were tested in accordance with ISO 5840-3:2021 guidelines for in vitro pulsatile flow testing for heart valve substitutes implanted by transcatheter techniques.[9] Explants were sutured in a holder fabricated from silicone with Shore hardness 30A. For 1 TAV explant still implanted within the surgical valve (sample 1), the index bioprosthesis was sutured in a silicone gasket with Shore hardness 40A, and silicone was applied between the frame and the holder to eliminate paravalvular leakage at the explant/holder interface. The compliance of the holders was chosen in accordance with ISO 5840-3:2021[9] and based on published data on tissue

compliance.[10-12] TAV explants were fixtured in the holder diameters that were representative of the indicated patient annular range per manufacturer recommendations and instructions for use. Each explant was sutured at 6 equidistant locations around the circumference of the inflow of the frame to the holder.

The test medium used during hydrodynamic testing was a 37 ± 2°C buffered saline solution (0.9 ± 0.2% sodium chloride). Measurements were based on average results taken from 10 consecutive cardiac cycles. Pulsatile flow performance was tested at a nominal rate of 70 ± 1 beats/min, systolic duration of 35% ± 5%, mean aortic pressure of 100 ± 2 mm Hg, and cardiac output of 5.0 ± 0.1 L/min. Mean gradient (mm Hg), effective orifice area (EOA) (cm^2), and regurgitant fraction (%) were quantified. A regurgitant fraction of >20% is considered significant in accordance with ISO 5840-3 standard. The regurgitant fraction represents the total regurgitant volume expressed as a percentage of the stroke volume. The EOA was derived from the continuity equation as previously described in ISO 5840-3,[9] where $q_{V_{RMS}}$ is the root mean square flow in mL/s, Δp is the mean pressure difference (measured over the positive differential pressure period of the forward flow phase) in mmHg, and ρ is the density of the test fluid in g/cm^3. The pressure gradient was calculated across the entire systolic cycle.

$$EOA = \frac{q_{V_{RMS}}}{51.6 \text{ x } \sqrt{\frac{\Delta p}{\rho}}}$$

A visual and photographic assessment was performed both pre- and post-hydrodynamic testing to ensure that there was no dislodgement of debris following bench testing of the explanted TAVs. All 14 explants were stratified based on their hydrodynamic mean gradient, with sample 1 having the highest transvalvular mean gradient and sample 14 having the lowest mean gradient.

PRE-EXPLANT ECHOCARDIOGRAPHY. The last transthoracic echocardiogram performed before TAV explantation was obtained from each clinical institution, when available. Only echocardiograms within 3 months before TAV explantation were included. All echocardiography data were site-reported. Echocardiographic parameters were compared with the hydrodynamic parameters measured ex vivo.

MORPHOLOGICAL APPEARANCE AND LEAFLET KINEMATICS. All TAV explants were visually assessed based on high-resolution photography with a digital microscope (Keyence) at a prespecified magnification and fixed camera height to make a qualitive assessment of leaflet calcification. Optical inspection was performed by 2 independent analysts including a vascular biologist and an interventional cardiologist with expertise in explanted bioprosthetic valves.

High-speed video from hydrodynamic testing was also performed to assess leaflet kinematics in terms of opening dynamics and leaflet coaptation from the outflow and inflow views. Specific to this study, we sought to describe restricted and asynchronous leaflet motion given the focus on TAV degeneration. All samples were placed in the valve holder with a reproducible orientation. The 3 leaflets were labeled with leaflet 1 (L1) at the 12 o'clock position, leaflet 2 (L2) at the 4 o'clock position, and leaflet 3 (L3) at the 8 o'clock position.

TAV CALCIFICATION QUANTIFICATION BY MICRO-CT. Quantitative assessment of leaflet calcium burden was assessed using micro-computed tomography (CT) imaging (North Star Imaging X5000). Calcium quantification on the leaflets was assessed using volume measurement of the 3-dimensional, reconstructed explanted TAVs. Reconstruction was performed in Mimics software, version 25.0 (Materialise). The analysis of calcification distribution was performed in 3-Matic software, version 17.0 (Materialise).

RELATIONSHIP OF FUNCTIONAL STENOSIS AND BURDEN OF TAV CALCIFICATION. An ex vivo hydrodynamic mean gradient of ≥20 mm Hg was defined in this study as functional stenosis. This is based on recommendations from the American Society of Echocardiography and the American College of Cardiology, which define an echocardiographic-derived Doppler mean gradient in stented biological valves of ≥20 mm Hg as a possible or significant stenosis.[13]

HISTOLOGICAL ANALYSIS. Following hydrodynamic testing and micro-CT imaging, TAV explants were treated with 100 mL of Cal-Ex II (Fisher CD511) for 24 hours. Subsequently, leaflets were removed from the TAV stent, marked with tissue dye, and processed for paraffin embedding. Samples were cut into 2- to 4-mm sections and embedded in paraffin in a cross-sectional orientation, yielding ~8 cross-sections per leaflet. Paraffin sections of 4-μm thickness were used for histology. Hematoxylin and eosin staining and Movat's pentachrome staining were used to assess overall pathology on high-resolution slide images using ImageScope software (Leica Biosystems) for slides scanned with an Aperio slide scanner. Sample 4 was not available for histological assessment.

TABLE 1 Clinical Characteristics of the 14 Transcatheter Aortic Valve Explants

Sample	Valve Type	Reason for Explantation (Degenerative Mechanism)	Sex	Age at the Time of Explantation, y	Time From Implantation to Explantation	Valve-in-Valve
1	26-mm SAPIEN 3	SVD (AS)	Male	68	4 y 1 mo	25-mm Perimount Magna as index valve
2	25-mm Lotus	SVD (AS)	Male	77	2 y 9 mo	No
3	34-mm Evolut R	SVD (AS)	Male	71	5 y 1 mo	No
4	23-mm Evolut R	SVD (AS)	Female	63	4 y 3 mo	Index valve not available
5	29-mm Evolut R	SVD (AS)	Male	60	5 y 11 mo	29-mm Evolut R as index valve
6	29-mm SAPIEN 3	SVD (AS)/endocarditis	Female	81	5 y 4 mo	No
7	23-mm Evolut PRO+	Endocarditis	Female	85	8 mo	No
8	29-mm CoreValve	SVD (AS)	Female	73	4 y 9 mo	No
9	23-mm Evolut PRO	NSVD (PPM)	Male	78	3 y 3 mo	23-mm Mitroflow as index valve
10	29-mm Evolut PRO	NSVD (PVL)/endocarditis	Male	79	1 y 7 mo	No
11	29-mm SAPIEN 3	Other (transplant for ischemic cardiomyopathy)	Male	63	4 y 2 mo	No
12	29-mm Evolut PRO+	NSVD (valve migration)	Male	82	2 wks	No
13	29-mm CoreValve	SVD (central AR)/NSVD (pannus)	Male	Not available	1 y 1 mo	No
14	31-mm CoreValve	NSVD (pannus)	Male	66	3 y 4 mo	No

AR = aortic regurgitation; AS = aortic stenosis; NSVD = nonstructural valve dysfunction; PPM = patient-prosthesis mismatch; PVL = paravalvular leak; SVD = structural valve deterioration.

STATISTICAL ANALYSIS. Continuous variables are presented as median with (Q1-Q3), and categorical variables as counts and percentages. Comparisons between continuous variables were performed using nonparametric rank-based (Wilcoxon) tests. Correlations were assessed using linear regression analysis and examined with a calculation of Spearman coefficients. Results were considered statistically significant when the P value was <0.05. Statistical analyses were performed using MINITAB software, version 20.1.3 (Minitab).

RESULTS

CLINICAL, PROCEDURAL AND ECHOCARDIOGRAPHIC CHARACTERISTICS OF TAV EXPLANTS. Clinical characteristics of the 14 TAV explants at the time of surgical explantation are found in Table 1. The median patient age at explantation was 73.0 years (Q1-Q3: 64.5-80.0 years), and 4 (30%) were women. The median time from TAVR to surgical explantation was 4 years and 1 month (1 year and 5 months to 4 years and 11 months). Of all samples, 71% (10/14) were explanted >2 years after TAVR implantation, with 1 patient (7%) requiring explantation <30 days of TAVR due to valve migration (sample 12). After excluding endocarditis, pannus, and migration, the median time from TAVR to surgical explantation remained similar at 4 years and 3 months (3 years and 8 months to 5 years and 2 months).

Surgical indications for TAVR explant from the heart teams included: SVD causing AS (50%, 7/14), patient-prosthesis mismatch, pannus or valve migration causing NVSD (22%, 3/14), endocarditis (7%, 1/14), a mix of SVD/NSVD/endocarditis (14%, 2/14), and transplantation (7%, 1/14) (Table 1). The median time to SVD was 4 years and 9 months (4 years and 1 month to 5 years and 4 months). TAV explantation after a valve-in-valve procedure was performed in 4 cases (29%). Sample 1 was implanted within a Magna surgical bioprosthesis (Edwards Lifesciences), sample 9 within a Mitroflow surgical bioprosthesis (Sorin Group), and sample 5 within an Evolut R TAV. The index bioprosthesis type was not available for sample 4. Of these 4 valve-in-valve TAV explants, samples 1 and 5 were explanted with the index bioprosthesis still attached to the TAV explant.

Pre-explantation transthoracic echocardiographic parameters are shown in Table 2, when available. Echocardiographic mean gradients were 40.0 mm Hg (15.0-44.0 mm Hg), 33.0 mm Hg (6.0-46.0 mm Hg), and 40 mm Hg for the CoreValve/Evolut, SAPIEN 3, and Lotus TAVs, respectively.

HYDRODYNAMIC ASSESSMENT AND COMPARISON TO PRE-EXPLANTATION ECHOCARDIOGRAMS. Transvalvular gradients measured ex vivo were 32.0 mm Hg (13.4-44.9 mm Hg), 37.5 mm Hg (19.9-76.2 mm Hg), and 75.6 mm Hg for the CoreValve/Evolut, SAPIEN 3, and Lotus TAVs, respectively. As shown in Tables 1 and 2, all 14 TAV explants were stratified in descending order based on their hydrodynamic mean gradient to allow comparison to the total leaflet calcium volume. Based on the American Society of Echocardiography

TABLE 2 Echocardiographic and Ex Vivo Characteristics of the 14 Transcatheter Aortic Valve Explants

		Pre-Explantation TTE		Hydrodynamic Testing			Micro-CT	
Sample	Valve Type	Mean Gradient, mm Hg	Aortic Regurgitation Severity	Mean Gradient, mm Hg	EOA, cm^2	Regurgitant Fraction, %	Calcium Volume, mm^3	Leaflet Kinematics
1	26-mm SAPIEN 3	33	None	76.2	0.73	4.9	0	L1 and L2 fixed L3 opening
2	25-mm Lotus	40	Moderate	75.6	0.73	6.1	77.9	L1 torn L2 with restricted motion L3 fixed
3	34-mm Evolut R	41	Moderate	73.6	0.70	40.4	603.0	Severely restricted motion of all leaflets L1 with perforation
4	23-mm Evolut R	79	Not available	51.9	0.90	5.4	77.9	Restricted motion of all leaflets
5	29-mm Evolut R	40	Moderate-severe	42.5	0.98	31.1	336.1	Restricted motion of all leaflets
6	29-mm SAPIEN 3	46	None	37.5	1.09	16.6	0	Asynchronous opening of L1 and L2 L3 fixed
7	23-mm Evolut PRO+	15	Moderate	34.4	1.11	4.5	0	Restricted and asynchronous motion of all leaflets
8	29-mm CoreValve	Not available	Not available	32.5	1.16	21.9	246.8	Restricted and asynchronous motion of all leaflets
9	23-mm Evolut PRO	44	None	31.4	1.18	2.6	0	Asynchronous motion of L1 and L3 L2 fixed
10	29-mm Evolut PRO	Not available	Not available	23.7	1.37	3.9	0	Restricted motion of L1 and L3 L2 opening
11	29-mm SAPIEN 3	6	Not available	19.9	1.53	7.0	0	L1 opening Severely restricted motion of L2 and L3
12	29-mm Evolut PRO+	4	None	13.6	1.84	2.6	0	L1 and L3 opening L2 fixed
13	29-mm CoreValve	Not available	Not available	12.9	1.93	2.8	0	Restricted motion of L1 L2 and L3 opening
14	31-mm CoreValve	17	Not available	8.8	2.49	19.9	1.4	Minimal restriction of L3 L1 and L2 opening

CT = computed tomography; EOA = effective orifice area; L1/2/3 = leaflet 1/2/3; TTE = transthoracic echocardiography.

criteria, functional stenosis was present in 79% (11/14) of TAV explants. All 7 TAV explants labeled as having SVD-AS as the primary reason for explantation were found to have an ex vivo transvalvular gradient of >20 mm Hg.

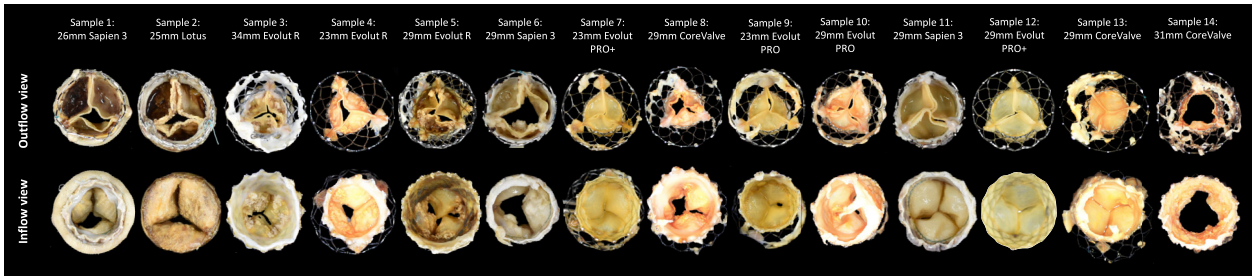
Ex vivo EOAs were 1.17 cm^2 (0.96-1.86 cm^2), 1.09 cm^2 (0.73-1.53 cm^2), and 0.73 cm^2 for the CoreValve/Evolut, SAPIEN 3, and Lotus TAVs, respectively. Hydrodynamic regurgitant fractions were 4.95% (2.75%-24.2%), 7.0% (4.9%-16.6%), and 6.1% for the CoreValve/Evolut, SAPIEN 3, and Lotus TAVs, respectively. Of all samples, 21% (3/14) exceeded the 20% regurgitation fraction threshold specified by ISO 5840-3 standard. Two of these 3 TAV explants were reported to have moderate or severe aortic regurgitation from the pre-explantation echocardiograms.

No significant difference was found between ex vivo hydrodynamic gradients (37.5 mm Hg; 19.9-73.6 mm Hg) and echocardiographic gradients (40.0 mm Hg; 15.0-44.0 mm Hg; $P = 0.21$). Although not statistically significant, hydrodynamic and echocardiographic gradients were observed to be weakly correlated ($r = 0.48$; $P = 0.14$).

MORPHOLOGICAL APPEARANCE AND LEAFLET KINEMATICS. Figure 1 shows the photographic assessment of the 14 TAV explants and the morphological burden of leaflet calcification. As shown in Figure 2 and Video 1, all 14 TAV explants had restricted motion or asynchronous leaflet motion affecting at least 1 leaflet. Eight (57%) explants had no leaflet calcification (Table 2), but all had significantly impaired leaflet kinematics affecting at least 1 leaflet.

LEAFLET CALCIUM BURDEN. Six explanted TAVs were found to have leaflet calcification (162.4 mm^3; 58.8-603.0 mm^3) and 8 TAVs had no calcium by micro-CT quantification (Table 2, Figure 3). Of the 6 calcified TAV explants, samples 3 and 5 had severe calcification (>300 mm^3) with nodular calcium present on the inflow and outflow surfaces of the leaflets. Samples 2, 4, and 8 had moderate calcification (50-300 mm^3), whereas sample 14 had minor calcification

FIGURE 1 Morphological Appearance of Explanted Transcatheter Aortic Valves



Photographic assessment of the 14 transcatheter aortic valve explants and morphological burden of leaflet calcification.

(<50 mm^3) only affecting 1 leaflet (Figure 3). Five of the 6 calcified TAV explants were reported to have SVD-AS to be the main reason for TAV explantation.

HISTOLOGICAL ASSESSMENT. Histopathology of representative images is shown in Figure 3 for each TAV leaflet available for analysis. Overall, histological findings were consistent with micro-CT analysis; no samples without calcium on micro-CT were found to have calcification on histology, and conversely, no samples were found to have calcification on histology not noted on micro-CT.

Two samples showed extensive calcification throughout all 3 leaflets on histology, which correlated with samples with >300 mm^3 of calcification on micro-CT. Three samples showed moderate calcification, corresponding to TAVs with calcification noted between 50 and 300 mm^3 on micro-CT. Finally, we found 1 sample to have minor calcification, in line with micro-CT calcium finding of <50 mm^3. Three samples showed evidence of active endocarditis or a history of endocarditis on histology, consistent with clinical history (Table 1). Pannus was noted in line with gross pathology and clinical reason for explantation.

RELATIONSHIP OF HYDRODYNAMIC MEAN GRADIENT AND BURDEN OF LEAFLET CALCIFICATION. Hydrodynamic mean gradients were 47.2 mm Hg (26.6-74.1 mm Hg) and 27.6 mm Hg (15.2-36.7 mm Hg) for the 6 calcified and 8 noncalcified explants, respectively. There was no significant difference in gradients between calcified and noncalcified TAV explants ($P = 0.28$) (Figure 4A).

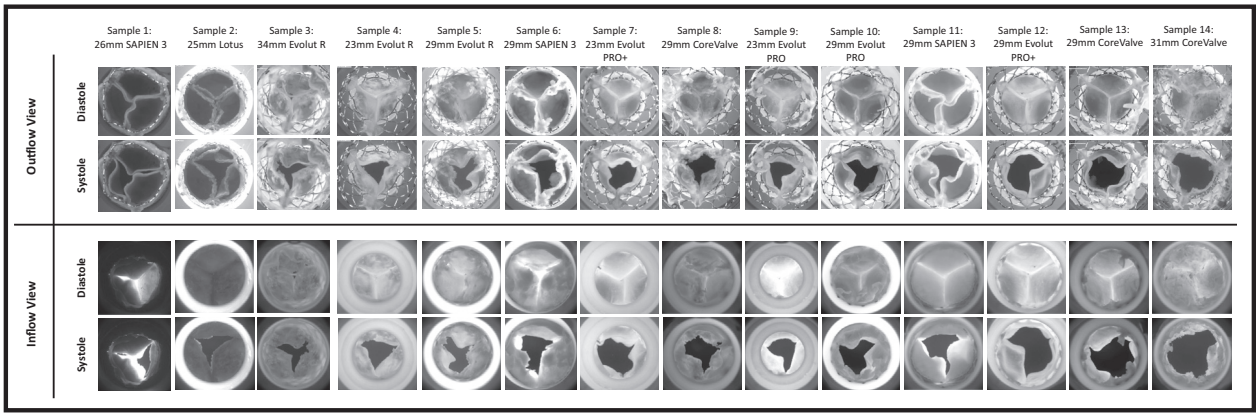
When evaluating the entire explanted cohort, there was a weak but nonsignificant association between hydrodynamic mean gradient and leaflet calcification ($r = 0.42$; $P = 0.14$) (Figure 4B). When evaluating the 10 self-expanding TAV explants, however, a moderate association between hydrodynamic mean gradient and burden of calcification was found ($r = 0.64$; $P = 0.05$) (Figure 4C).

RELATIONSHIP OF HYDRODYNAMIC MEAN GRADIENT AND STENOSIS. When evaluating the 11 TAV explants having functional stenosis, defined as a hydrodynamic mean gradient ≥20 mm Hg, hydrodynamic mean gradient and leaflet calcification were found to be weakly correlated, although this was not statistically significant ($r = 0.43$; $P = 0.19$) (Figure 5A). A similar result was obtained when examining the 7 TAV explants having SVD-AS ($r = -0.16$; $P = 0.73$) (Figure 5B).

DISCUSSION

In this first-of-a-kind study, we demonstrated the impact of different mechanisms of TAV degeneration on valve function and leaflet kinematics. To our knowledge, this is the first and largest report of hydrodynamic testing, multimodality imaging, and histological analysis performed on explanted degenerated TAVs. We identified several key findings and implications for future clinical practice (Central Illustration). First, hydrodynamic bench testing allowed us to evaluate the performance and leaflet kinematics of explanted degenerated TAVs under controlled physiological conditions. Second, 8 of the 14 TAV explants had no leaflet calcification by micro-CT quantification and histology, but all had impaired leaflet kinematics affecting at least 1 leaflet. Third, no significant difference in mean gradients was demonstrated between calcified and noncalcified TAV explants. Fourth, calcium volume had a nonsignificant association with the mean gradient, which suggests

FIGURE 2 Leaflet Kinematics From Hydrodynamic Testing at Systole and Diastole



Photographic assessment of the 14 transcatheter aortic valve explants during pulsatile flow testing.

that functional stenosis can occur in TAVs in the absence of significant leaflet calcification. Thus, similar to bioprosthetic surgical valves, noncalcific and calcific degenerative mechanisms may lead to TAV stenosis and dysfunction.

Despite the availability of bioprosthetic surgical valves for several decades, a consistent definition of SVD or the mechanisms leading to TAV degeneration are poorly understood. SVD refers to failure of the intrinsic properties of the bioprosthetic valve biomaterials.[5] Typically, SVD is associated with calcification and several cellular mechanisms leading to the pathogenesis of leaflet calcification.[5] However, the assumption that calcification is the only cause in all cases of valve dysfunction is incorrect.[14] It has been shown that up to 25% of patients who have redo surgery attributed to SVD of the surgical valve present with no or minimal leaflet calcification.[4] Noncalcific mechanisms of dysfunction and degeneration are poorly understood, particularly in TAVs, where there are few studies of degeneration.[7,15] These mechanisms are felt to be multifactorial, and can involve leaflet thrombosis, microscopic structural degeneration and delamination, infection, fibrosis, protein infiltration, and inflammation. However, these studies have examined earlier explants, whereas the cohort in this study represents an analysis of longer-term implantation.

There may also be an association between calcific and noncalcific mechanisms of SVD. In our series, we did not demonstrate a significant strong association with the degree of calcification and ex vivo mean gradients for the total cohort. Of note, explanted TAVs, particularly those explanted for degeneration, are rare, and it is possible that with more samples, a correlation between calcium burden and functional stenosis would be more evident. Indeed, there was a moderate association noted between calcium burden and hydrodynamic gradients with the self-expanding TAVs alone. However, similar to bioprosthetic surgical valves, there are mechanisms of TAV degeneration that can occur in the absence of calcification, which cause stenosis. Hydrodynamic testing performed in this study provides insights into leaflet kinematics in noncalcific degeneration, and this has the future potential to define potential etiologies that impact valve function.

The mechanism of failure also has implications for both the identification and treatment of a failed TAV. Multimodality imaging, which includes echocardiography and computed tomography (CT), often focuses on calcification as a mechanism of failure.[16,17] The identification of noncalcific mechanisms of TAV degeneration may be more challenging, and patients may potentially be misdiagnosed. In this work, 1 patient had an Evolut TAV explanted based on a suspicion of patient-prosthesis mismatch (sample 9). However, post-explantation testing demonstrated severe leaflet restriction and asynchronous leaflet motion associated with an elevated gradient, but no leaflet calcification. This suggests that the potential mechanism of failure was SVD due to a noncalcific etiology. Similarly, 2 of the 3 SAPIEN 3 TAVs were explanted due to SVD (samples 1 and 6), but impaired leaflet kinematics appeared to be caused by a noncalcific mechanism. Appropriate identification of patients with SVD and the etiology will be crucial to their timely diagnosis and treatment.

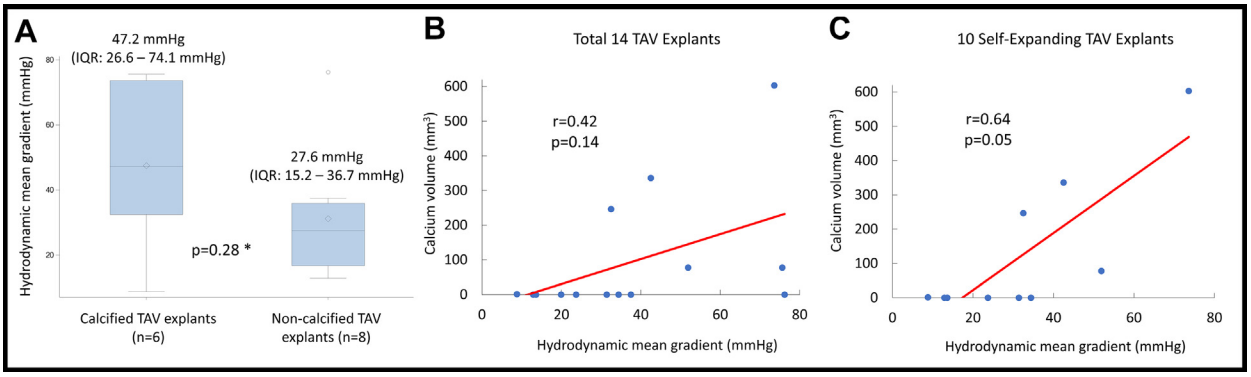
FIGURE 3 Quantification of Calcium Burden, Hydrodynamic Mean Gradient, and Histological Evaluation

Sample Number	Micro-CT Images	Calcium Volume (mm^3)	Hydrodynamic Mean Gradient (mmHg)	Histology Cuts: Leaflet 1 / Leaflet 2 / Leaflet 3	Primary Pathological Finding
Sample 1: 26mm Sapien 3	No Calcium	0	76.2		Leaflet thrombosis
Sample 2: 25mm Lotus		77.9	75.6		Moderate calcification
Sample 3: 34mm Evolut R		603.0	73.6		Extensive calcification
Sample 4: 23mm Evolut R		77.9	51.9	Not Available	
Sample 5: 29mm Evolut R		336.1	42.5		Extensive calcification
Sample 6: 29mm Sapien 3	No Calcium	0	37.5		Active Endocarditis
Sample 7: 23mm Evolut PRO+	No Calcium	0	34.4		Recent Endocarditis
Sample 8: 29mm CoreValve		246.8	32.5		Moderate - Extensive calcification
Sample 9: 23mm Evolut PRO	No Calcium	0	31.4		Microscopic intrinsic degeneration
Sample 10: 29mm Evolut PRO	No Calcium	0	23.7		Organizing inflammatory – History of endocarditis
Sample 11: 29mm Sapien 3	No Calcium	0	19.9		Microscopic degeneration with commissural fibrosis
Sample 12: 29mm Evolut PRO+	No Calcium	0	13.6		Microscopic thrombus
Sample 13: 29mm CoreValve	No Calcium	0	12.9		Pannus, microscopic intrinsic degeneration
Sample 14: 31mm CoreValve		1.4	8.8		Minor calcification, pannus, microscopic degeneration

Stent Leaflets Calcium — 300 µm Calcification Pannus

Micro-computed tomography (micro-CT) imaging, total calcium volume quantification, hydrodynamic mean gradient, and histological assessment of the 14 transcatheter aortic valve (TAV) explants. Sample 5, a TAV-in-TAV case, was imaged with the 2 TAV frames.

FIGURE 4 Association of Hydrodynamic Mean Gradient and Burden of Leaflet Calcification

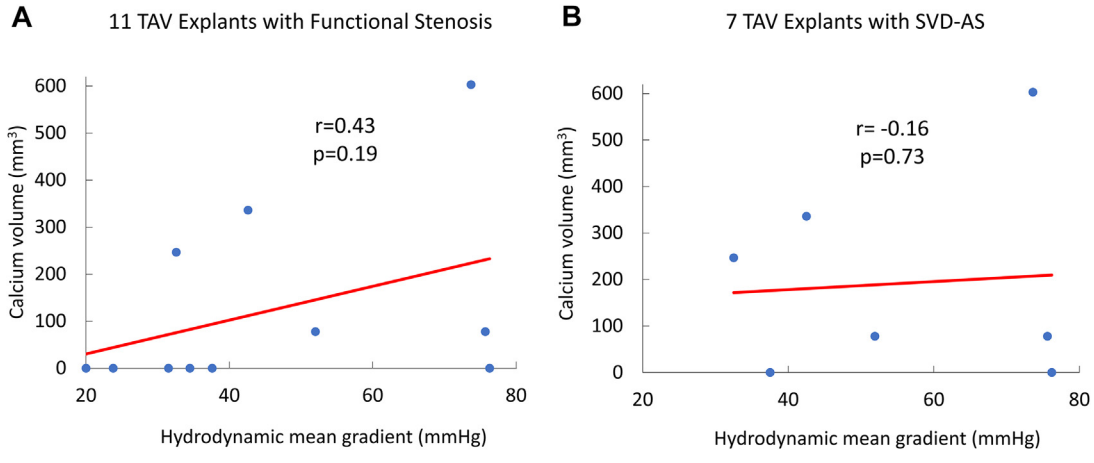


Association of hydrodynamic gradient and burden of leaflet calcification for (A) calcified vs noncalcified transcatheter aortic valve (TAV) explants, (B) the entire explant cohort, and (C) the 10 self-expanding TAV explants. *Wilcoxon rank-based test.

Beyond appropriate and timely diagnosis of SVD, there are also implications for patient management. Despite promising data from multiple clinical trials and isolated series demonstrating low rates of SVD up to 5 years and even 10 years in some cases,[18-20] it is important to note that the majority of these patients were elderly. Therefore, it is possible to anticipate that with the rise in TAVR procedures, particularly among patients aged 70 years or younger, the number of individuals experiencing TAV degeneration requiring redo-TAVR will increase.[21] In this study, the median patient age at explant for patients with SVD was 71.0. years (63.0-77.0 years), and the median time to SVD was 4 years and 9 months (4 years and 1 month to 5 years and 4 months). A calcified TAV may cause potential issues with redo-TAVR related to expansion and function of the new TAV. The implications for performing redo-TAVR in the presence of noncalcific TAV degeneration mechanisms are unknown.

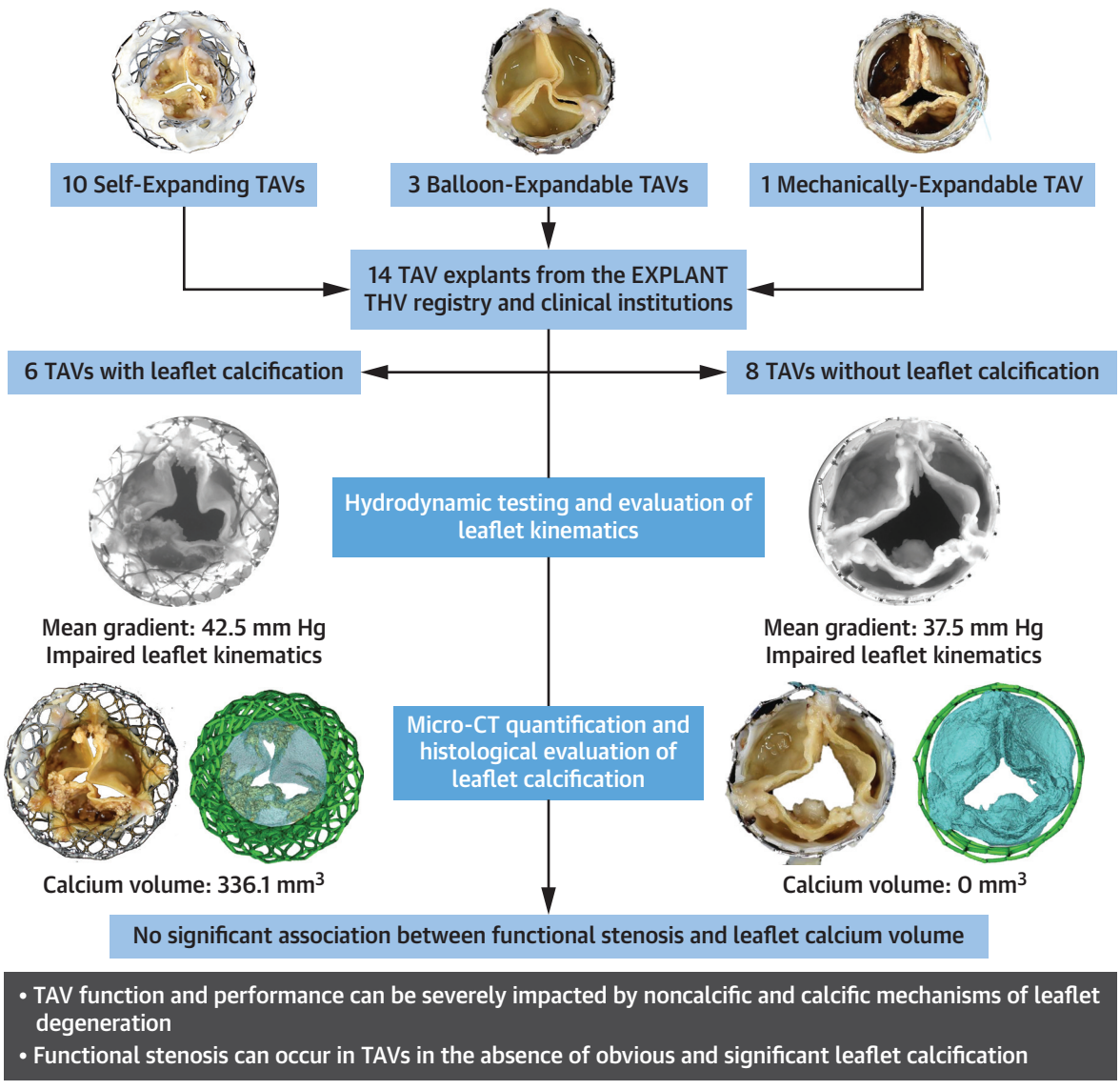
STUDY LIMITATIONS. Foremost, given the major challenge that represents explant collection, the relatively small number of TAV explants analyzed in this series is a limitation. Even if this study represents

FIGURE 5 Association of Hydrodynamic Mean Gradient and Stenosis



Association of hydrodynamic gradient and burden of leaflet calcification for (A) the 11 transcatheter aortic valve (TAV) explants with functional stenosis, and (B) the 7 TAV explants with structural valve deterioration from aortic stenosis (SVD-AS).

CENTRAL ILLUSTRATION **Hydrodynamic Assessment of Explanted Transcatheter Aortic Valves: Insights Into Valve Degeneration**



Sathananthan J, et al. J Am Coll Cardiol Intv. 2024;17(11):1340–1351.

Transcatheter aortic valve (TAV) function and performance can be severely impacted by noncalcific and calcific mechanisms of leaflet degeneration. Functional stenosis can occur in TAVs in the absence of obvious and significant leaflet calcification. CT = computed tomography.

the first and largest experience to date of ex vivo hydrodynamic testing and multimodality imaging of explanted TAVs, the relative lack of power of some statistical analyses could leave some questions unanswered. Certainly, further studies will be useful in advancing knowledge but will be dependent on explants continued collection in the EXPLANT THV Registry and by collaborating clinical institutions. It is also unknown what the total number of failed TAVs is at each center, which is challenging information to obtain as patients may have died from other causes or not been referred to the same center. Primary indications for TAV explantation were assessed independently by the heart team at each institution, which may have introduced patient selection biases. Additionally, a total of 6 explants had to be excluded due to excessive TAV frame damage caused by explant techniques rather than the nature of

degenerative changes. This is in alignment with challenges of other studies of TAV using explants, which note that samples obtained from surgical explantation (ie, samples of convenience) cannot be certain to represent the full spectrum of TAV pathology and dysfunction. Bench testing design does not fully replicate patient-specific physiological conditions in clinical practice, where flow and hemodynamic status are influenced by many additional parameters that are not accounted for in the present study. It is also possible that the explantation technique and storage protocols may have had an impact on hydrodynamic function and exact leaflet kinematics. However, testing was performed according to ISO 5840-3 guidelines that do not intend to recreate patient-specific in vivo conditions but rather aim at a standardized approach to assess valve function in isolation, thus eliminating patient-related confounding factors. These aspects are likely to explain the differences seen between in vivo and ex vivo transvalvular gradients.

It must also be noted that all but one TAV was explanted due to degeneration and abnormal clinical hemodynamic findings, and that no significant differences were found between hydrodynamic gradients and clinical echocardiographic data. Thus, although the exact degree of leaflet restriction might have been slightly different in vivo, the present analysis still offers insights into the general pattern of leaflet restriction even in the complete absence of leaflet calcification. This is consistent with the aim of this analysis: to assess valve function ex vivo relative to valve degeneration in a standardized fashion, opposed to replicating exact patient-specific parameters. Finally, patient-level data were limited, which impacted the ability to fully understand potential etiologies for TAV degeneration. Our study findings remain hypothesis-generating, and further in-depth analyses are warranted. Among these, clinical studies comparing calcification burden derived from clinical imaging such as CT to echocardiographic gradients are warranted in order to be able to quantify the nature and degree of degenerative changes in vivo.

CONCLUSIONS

This first-of-a-kind report combining hydrodynamic testing, multimodality imaging, and histological assessment on explanted failed TAV found that functional stenosis can occur to a similar extent in the absence of leaflet calcification. All TAV explants evaluated in this study presented with impaired leaflet kinematics affecting at least 1 leaflet. Other mechanisms of TAV dysfunction and degeneration beyond calcification alone will need to be further assessed. Our findings suggest that the impact of noncalcific mechanisms of TAV degeneration are not negligible and should be taken into consideration when evaluating longer-term TAV performance and lifetime management of AS.

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PERSPECTIVES

WHAT IS KNOWN? The etiology of TAV degeneration is not well understood, particularly noncalcific mechanisms. Different mechanisms of TAV degeneration may have a potential impact on diagnosis and repeat treatment in patients with TAV degeneration.

WHAT IS NEW? This is the first and largest report of hydrodynamic testing, multimodality imaging, and histological analysis performed on explanted failed TAVs. Hydrodynamic assessment and micro-CT imaging of TAV explants showed that functional stenosis can occur in TAVs in the absence of significant leaflet calcification. Similar to surgical bioprostheses, noncalcific and calcific degenerative mechanisms may lead to functional stenosis in TAVs.

WHAT IS NEXT? Future studies will be needed to better understand noncalcific mechanisms of TAV degeneration and will require a larger cohort, detailed histopathological and immunohistochemistry analyses, and clinical studies. The implications of noncalcific degeneration on diagnosis and treatment of degenerated TAVs will also be of importance.

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KEY WORDS calcification, degeneration and dysfunction, multimodality imaging, TAVR explantation, transcatheter aortic valve replacement

APPENDIX For a supplemental video, please see the online version of this paper.