Concomitant Transthyretin Amyloidosis and Severe Aortic Stenosis in Elderly Indian Population
A Pilot Study

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ABSTRACT

BACKGROUND Prevalence of both degenerative severe aortic stenosis (AS) and transthyretin cardiac amyloidosis (ATTR-CA) increases with age. Dual disease (AS þ myocardial ATTR-CA) occurs in significant proportion of patients undergoing surgical aortic valve replacement (SAVR).

OBJECTIVES This study aimed to determine the prevalence of ATTR-CA in severe AS in the Indian population, identify noninvasive predictors of its diagnosis, and understand its impact on prognosis.

METHODS Symptomatic severe AS patients aged ≥65 years undergoing SAVR were enrolled. ATTR-CA diagnosis was based on preoperative 99m-technetium pyrophosphate (PYP) scan and intraoperatively obtained basal interventricular septum biopsy for myocardial ATTR-CA, and excised native aortic valve for isolated valvular ATTR-CA. Primary amyloidosis was excluded by serum/urine protein electrophoresis with serum immunofixation.

RESULTS SAVR was performed in 46 AS patients (age 70 ± 5 years, 70% men). PYP scan was performed for 32 patients, with significant PYP uptake in 3 (n = 3 of 32, 9.4%), suggestive of myocardial ATTR-CA. On histopathological examination, none of the interventricular septum biopsy specimens had amyloid deposits, whereas 33 (71.7%) native aortic valves showed amyloid deposits, of which 19 (57.6%) had transthyretin deposition suggestive of isolated valvular amyloidosis. Noninvasive markers of dual disease included low myocardial contraction fraction (median [interquartile range], 28.8% [23.8% to 39.1%] vs 15.3% [9.3% to 16.1%]; P = 0.006), deceleration time (215 [144 to 236] ms vs 88 [60 to 106] ms; P = 0.009) and global longitudinal strain (-18.7% [-21.1% to -16.9%] vs -14.2% [-17.0% to -9.7%]; P = 0.030). At 1-year follow-up, 2 patients died (4.3%); 1 each in myocardial ATTR-CA negative and positive groups (3.4% vs 33.3%; P = 0.477).

CONCLUSIONS Dual disease is not uncommon in India. Isolated valvular amyloidosis in severe AS is much more common. (J Am Coll Cardiol Cardio Onc 2021;3:565–576) © 2021 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
The prevalence of both degenerative severe aortic stenosis (AS) and transthyretin (TTR) cardiac amyloidosis (ATTR-CA) increases with age (severe AS in ~3% of patients aged >75 years and ATTR-CA in ~25% of those >80 years) (1-3). Multiple studies suggest that ATTR-CA may act as a disease and prognosis modifier in patients with severe AS (4-7). The prevalence of ATTR-CA is notably higher in patients with severe AS, especially among those with low-flow, low-gradient (LflG) AS. Up to 6% of those undergoing surgical aortic valve replacement (SAVR) and 15% of those undergoing transcatheter aortic valve replacement (TAVR) have been shown to have ATTR-CA (5,6). Presence of ATTR-CA influences the management and clinical course of AS by distorting the severity assessment of AS and also by worsening the prognosis (5,8).

Whether this is by chance or there is a cause-and-effect relationship is not clear as of now. However, it appears that this coexistence leads to worsening of symptoms and a less-than-expected response to standard therapy. These patients tend to fare poorly as compared to those with lone AS, despite aortic valve replacement (TAVR or SAVR).

With the advent of TAVR, the number of patients undergoing aortic valve replacement (AVR) is increasing, thus making it pertinent that this group of patients with dual disease (AS + ATTR-CA) is identified preoperatively by noninvasive methods so that beyond AVR for severe AS, these patients are also considered for medications directed against the accumulating myocardial TTR deposits.

ATTR-CA is a disease with known racial and ethnic variations in its distribution and prevalence (9). Considering the lack of data regarding concomitant existence of ATTR-CA and severe AS in the Asian/Indian context, we planned this study to determine the prevalence rate of this dual disease in India, identify noninvasive predictors of its diagnosis, and study its impact on prognosis. Taking into consideration the burden of senile severe AS in India which merits AVR, even a small relative percentage would amount to a large absolute number of patients with ATTR-CA.

METHODS

This is a single-center prospective observational pilot study. The study protocol was approved by the institutional ethics committee (reference number: IECG-441/27.09.2018) and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. All patients gave written informed consent. We recruited all consecutive elderly patients (≥65 years of age) with severe AS being planned for SAVR at our center between December 2018 and March 2020. Patients who had suspicion of rheumatic etiology or those aged <65 years were excluded from the study.

Because no research work in this field was previously reported from low and low-middle-income countries such as ours, this was designed as a pilot study, targeting a sample size of 50 patients. By the time the coronavirus disease-2019 pandemic began, 46 patients had been recruited, and the study was stopped at that point. Figure 1 outlines the schematic flow of patients through the study. All patients underwent routine preoperative investigations, including blood tests, electrocardiogram (ECG), and basic echocardiographic study.

ECHOCARDIOGRAPHY. A detailed 2-dimensional (2D) transthoracic echocardiogram with tissue Doppler imaging and speckle strain imaging was performed (Phillips EPIQ 7C). All echo-based measurements were performed in accordance with the local lab protocols which are based on the 2017 American Society of Echocardiography (ASE) and European Association of Cardiovascular Imaging guidelines (10). Various cut-offs for echo-based values were derived from the expert consensus recommendations for multimodality imaging in cardiac amyloidosis endorsed by the American Society of Nuclear Cardiology (ASNC)/American Heart Association (AHA)/ASE (11). These included left ventricular ejection fraction (LVEF) ≤50%, myocardial contraction fraction (MCF) (ie, the ratio of stroke volume to myocardial volume) ≥30%, relative wall thickness (2·posterior wall thickness/ left ventricular [LV] internal diameter) ≥0.42, E/A ratio ≥1.5, deceleration time (dT) ≤150 ms, mitral annular tissue Doppler index (TDI) (mean of lateral + medial annulus values of S’, e’, a’) all ≤5 cm/s, right ventricular S’ (lateral annulus) ≤10 cm/s, absolute mean global longitudinal strain (GLS) ≤−15%. Relative
Apical strain was calculated as: \[
\frac{\text{mean apical strain}}{\text{mean basal strain} + \text{mean mid strain}}
\], and relative apical strain sparing was noted if this value was > 1.

**Radionuclide scintigraphy.** Standard dose of 99m-technetium pyrophosphate (PYP) for cardiac imaging (10 to 20 mCi) was injected intravenously. Image acquisition was performed on a GE Infinia Hawkeye 4 dual-head gamma camera mounted with low-energy all-purpose (LEAP) collimators with parallel holes. At 1-hour post-injection, both planar and single-photon emission computed tomography (SPECT) (rest scans) were acquired with heart in the center of field of view. If blood pool activity was suspected in the images at 1 hour, the scan was repeated at 3 hours post-injection. Time for each projection was 15 to 20 seconds leading to a total scan duration of approximately 20 to 25 minutes. The same imaging procedure was repeated at 3 hours if required. The scans were visually assessed qualitatively (heart-to-bone ratio, Perugini score) and semiquantitatively (heart-to-contralateral-lung [H:CL] uptake, ie, the H:CL ratio). Based on the expert consensus recommendations for multimodality imaging in cardiac amyloidosis endorsed by the ASNC/AHA/ASE, Perugini grade II or III, and H:CL ratio ≥1.50 were considered as significantly positive for diagnosis of myocardial ATTR-CA (11). Perugini grade I was considered borderline positive in this study.

**Endomyocardial biopsy.** During SAVR, a tissue sample was obtained under direct vision by performing a LV endomyocardial biopsy from the basal segment of the interventricular septum (IVS). Also, the excised native aortic valve was sent for histopathological examination.

**Histopathological examination.** Tissue samples thus obtained were formalin fixed. Congo red staining was performed to look for apple green birefringence suggesting amyloid deposits. Supplemental Table 2 summarizes the histopathological grading of IVS tissue and aortic valve based on hematoxylin and eosin staining and Congo red staining. For patients in whom the PYP scan was positive, the biopsy specimens were independently reviewed by a second senior pathologist.

For subtyping the amyloid detected on congo red staining, immunohistochemistry (IHC) using specific...
antibodies against TTR protein was performed on samples showing apple green birefringence under polarized light microscope. TTR-specific antibodies (Invitrogen) were used in a dilution of 1:50. Protocol for IHC analysis including tissue fixation, heat-induced antigen (epitope) retrieval, antibody binding, detection under microscope, and counterstaining is summarized in Supplemental Figure 1.

MONOCLONAL PROTEIN DETECTION. Primary light chain cardiac amyloidosis and monoclonal gammopathy of unknown significance (MGUS) were ruled out in patients diagnosed to have amyloidosis by performing serum protein electrophoresis, urine protein electrophoresis, and serum immunofixation. A bone marrow biopsy was planned to rule out monoclonal cell expansion in cases where M-band was detected in either of these investigations.

All these tests, including 2D echocardiography, PYP scan, and histopathological analysis were performed independent of each other and in a blinded fashion. For echocardiography, effective aortic orifice area, TDI, and strain imaging, findings were confirmed by a second reviewer. PYP scan and histopathological examination results were also independently verified by a second examiner.

STATISTICAL METHODS. Statistical analysis was performed using SPSS V27 software. For continuous variables, median (25th and 75th percentiles [interquartile range]) was calculated and compared using Wilcoxon rank sum test. For categorical variables, frequency and percentage were calculated. Categorical variables were compared using chi square or Fisher exact test. A P value <0.05 was considered statistically significant.

RESULTS

Seventy-two patients aged ≥65 years who were symptomatic with severe AS were screened, of which 46 underwent SAVR and are included in the present analysis. The remaining 26 patients were excluded due to various reasons, including refusal to undergo AVR (n = 15), loss to follow-up preoperatively (n = 5), death before SAVR (n = 4), and suspected rheumatic heart disease (n = 2) (Central Illustration). These 46 patients underwent the protocol-mandated preoperative investigations and a biopsy from the IVS was obtained intraoperatively. Their baseline characteristics are presented in Table 1. The mean age of the group was 70 ± 5 years, 70% were men, and majority of the patients were in New York Heart Association functional class II (76%). None of the patients had carpal tunnel syndrome, ruptured biceps tendon, or atrial fibrillation.

2D ECHOCARDIOGRAPHY. All the patients underwent a detailed 2D transthoracic echocardiogram. The mean LVEF was 55.6 ± 13%. Twelve patients (26%) had LV systolic dysfunction (LVEF ≤50%). The mean indexed aortic valve area was 0.37 ± 0.11 cm²/m² and the mean gradient was 56.5 ± 21 mm Hg. Eight patients (17.4%) had LfLg AS. Details are provided in Table 1.

HISTOPATHOLOGY. Congo red staining. IVS biopsy and excised native aortic valve were obtained from all 46 patients undergoing SAVR. Both the tissue samples were subjected to histopathological examination per protocol. Amyloid deposition was suspected on hematoxylin and eosin staining (Figure 2A). This was confirmed on Congo red staining, under light microscope (Figure 2B) and under polarized microscope (Figure 2C). In the IVS biopsy specimens, none of the samples showed positivity on Congo red staining (Figure 3A). In the aortic valve specimens, 33 (71.7%) had positive uptake with Congo red stain and showed a negative apple green birefringence on polarized microscopy. Amyloid deposition was primarily observed adjacent to calcific deposits. These 33 valves were subjected to IHC to look specifically for TTR deposition.

IHC. Staining with TTR-specific antibodies was performed on the tissue specimens with Congo red positivity. Because none of the septum biopsy samples showed amyloid deposition, IHC was performed only on the aortic valves that were Congo red-positive. Nineteen of 33 valves (58%) showed uptake of TTR antibodies. Of the total 46 patients, isolated valvular amyloidosis was noted in 33 (72%), and isolated valvular TTR amyloidosis was observed in 19 (41%).

RADIONUCLIDE SCINTIGRAPHY. Of the total 46 patients, PYP imaging was performed in 32 patients (70%). Qualitatively, the most common Perugini grade was 0, noted in 23 of 32 patients (71.9%), followed by grade I (n = 6 of 32, 18.8%). Perugini grades II or III were observed in 3 patients (9.4%, 95% confidence interval: 2.0% to 25.0%). Different Perugini grades 0, I, II, and III are shown in Figure 4. Quantitatively, the mean H:CL ratio was 1.28 ± 0.14. Characteristics of the study cohort as distributed between PYP grades 0 or I versus grades II or III are presented in Table 1. Patients with grade II or III uptake were considered to have myocardial TTR amyloidosis; this group had significantly low MCF (36 ± 17% vs 14 ± 4%; P = 0.020), deceleration time (207 ± 80 ms vs 85 ± 23 ms; P = 0.003) and GLS (-19.3 ± 4.3% vs -13.6 ± 3.7%; P = 0.027).
Seventy-two patients with severe aortic stenosis were screened, of which 46 and 32 underwent surgical aortic valve replacement (SAVR) and 99m technetium pyrophosphate (PYP) scan, respectively. Significant PYP uptake was observed in 3 patients (n = 3 of 32; 9%), suggestive of myocardial transthyretin (TTR) cardiac amyloidosis (ATTR-CA). On histopathological examination, none of the interventricular septum biopsy specimens had amyloid deposits, whereas 33 (72%) native aortic valves showed amyloid deposits, of which 19 (58%) had TTR deposition suggestive of isolated valvular amyloidosis. At the 1-year follow-up examination, 2 had patients died (4.3%); 1 each in myocardial ATTR-CA-negative and -positive groups (3% vs 33%, \( P = 0.477 \)). CR = Congo red stain.
# TABLE 1  Procedural Details, Baseline Parameters, Histopathologic, and Radionuclide Scintigraphic Study Assessment of the Study Patients

| Procedural details, demographic profile, blood investigations, and ECG parameters | Overall Population (N = 46) | Isolated AS (n = 29) | Dual AS + ATTR-CA (n = 3) | P Value |
|---|---|---|---|---|
| Surgical aortic valve replacement | 46 (100) | 29 (100) | 3 (100) | 1.000 |
| Coronary artery bypass graft | 13 (28.3) | 9 (31.0) | 1 (33.3) | 0.935 |
| Permanent pacemaker implantation | 4 (8.7) | 3 (10.3) | 0 (0) | 0.558 |
| Median age (IQR), y | 69.5 (66.0-73.0) | 69.0 (66.0-73.0) | 70.0 (70.0-75.0) | 0.450 |
| Men | 32 (69.6) | 22 (75.9) | 1 (33.3) | 0.682 |
| NYHA functional class II | 35 (76.1) | 21 (72.4) | 2 (66.7) | 0.642 |
| Diabetes mellitus | 8 (17.4) | 6 (20.7) | 2 (66.7) | 0.089 |
| Hypertension | 17 (37.0) | 12 (41.4) | 1 (33.3) | 0.783 |
| Coronary artery disease | 14 (30.4) | 9 (31.0) | 2 (66.7) | 0.893 |
| Cerebrovascular disease | 1 (2.2) | 1 (3.4) | 0 (0) | 0.525 |
| Body surface area, m² | 1.6 (1.5-1.7) | 1.7 (1.6-1.7) | 1.7 (1.3-1.8) | 0.770 |
| Hemoglobin, gm/dL | 12.6 (11.6-13.6) | 12.5 (11.7-13.6) | 13.7 (11.2-15.0) | 0.460 |
| Creatinine, mg/dL | 0.9 (0.8-1.1) | 1.0 (0.9-1.1) | 0.8 (0.6-1.0) | 0.130 |
| Troponin I, pg/mL | 14.7 (8.1-29.3) | 14.4 (9.5-33.4) | 26.8 (16.3-53.1) | 0.280 |
| NT-proBNP, pg/mL | 261.2 (99.7-519.8) | 354.9 (94.4-916.5) | 517.7 (261.2-519.8) | 0.670 |
| Low voltage complexes | 4 (9.5) | 2 (7.4) | 1 (50.0) | 0.724 |
| Right bundle branch block | 3 (7.1) | 2 (7.4) | 0 (0) | 0.397 |
| E/e’ | 185.0 (109.0-229.0) | 215.0 (144.0-236.0) | 88.0 (22.8-38.1) | 0.009 |
| Indexed LA volume, mL/m² | 32.1 (27.1-41.4) | 32.4 (27.1-42.8) | 41.4 (25.4-41.7) | 0.190 |
| Septum thickness, mm | 14.1 (12.0-16.0) | 14.2 (12.4-16.9) | 14.5 (14.1-15.1) | 0.900 |
| Low mitral annular S’ (≤5 cm/s) | 15 (32.6) | 20 (69.0) | 3 (100) | 0.682 |
| Abnormal GLS (≤-15%) | 6 (13.0) | 4 (13.8) | 2 (66.7) | 0.020 |
| Relative apical strain | 0.7 (0.7-0.8) | 0.7 (0.7-0.8) | 0.7 (0.6-0.8) | 0.460 |
| Relative apical strain sparing | 0 | 0 | 0 | NA |
| Mean pressure gradient, mm Hg | 56.5 (45.0-70.0) | 60.0 (53.0-72.0) | 52.0 (36.0-70.0) | 0.330 |
| Indexed aortic valve area, cm²/m² | 0.4 (0.3-0.4) | 0.4 (0.3-0.4) | 0.2 (0.2-0.4) | 0.099 |
| High gradient severe AS | 38 (82.6) | 26 (89.7) | 2 (66.7) | 0.882 |
| Low-flow, low-gradient severe AS | 8 (17.4) | 3 (13.3) | 1 (33.3) | 0.882 |
| Classical low-flow, low-gradient AS | 4 (8.7) | 0 (0) | 1 (33.3) | 0.104 |
| Paradoxical low-flow, low-gradient AS | 4 (8.7) | 3 (10.3) | 0 (0) | 0.255 |

Histopathological and radionuclide scintigraphic study assessment

| Congo red positive – aortic valve | 33 (71.7) | 22 (75.9) | 3 (100) | 0.976 |
| IHC-positive – aortic valve | 19 (41.3) | 15 (51.7) | 2 (66.7) | 0.863 |
| Congo red-positive – IVS | 0 (0) | 0 (0) | 0 (0) | NA |
| IHC-positive – IVS | 0 (0) | 0 (0) | 0 (0) | NA |
| PYP – Perugini grade 0’ | 23 (71.9) | 23 (73.9) | 0 (0) | — |
| PYP – Perugini grade I’ | 6 (18.8) | 6 (20.7) | 0 (0) | — |
| PYP – Perugini grade II or III’ | 3 (9.4) | 0 (0) | 3 (100) | — |
| PYP – H:CL ratio’ | 1.3 (1.2-1.4) | 1.2 (1.2-1.3) | 1.6 (1.6-1.6) | — |
| 1-y mortality rate | 2 (4.3) | 1 (3.4) | 1 (33.3) | 0.477 |

Values are n (%) or median (interquartile range). *Of the total 46 patients who underwent SAVR, only 32 patients underwent a PYP scan (due to logistical constraints). Significant radiotracer uptake was seen in 3 patients (dual AS + ATTR-CA), and radiotracer uptake was not significant in the remaining 29 patients (isolated AS). 

2D = 2-dimensional; AS = aortic stenosis; ATTR-CA = transthyretin cardiac amyloidosis; dT = deceleration time; ECG = electrocardiogram; GLS = global longitudinal strain; IHC = immunohistochemistry; IVS = interventricular septum; LA = left atrial; LV = left ventricular; MCF = myocardial contraction fraction; NA = not available; NT-proBNP = N-terminal pro-B-type natriuretic peptide; NYHA = New York Heart Association; PYP = 99m-technetium pyrophosphate radionuclide scan; TDI = tissue Doppler index.
In the 3 patients with significant PYP uptake, histopathological examination of the aortic valve revealed all were Congo red-positive (amyloid positive), but only 2 of these stained positively with TTR antibodies on IHC (ie, TTR positive).

**MONOCLONAL PROTEIN DETECTION.** None of the patients showed presence of M band or other features suggestive of clonal cell expansion, thus ruling out primary light chain cardiac amyloidosis and monoclonal gammopathy of unknown significance.

**FOLLOW-UP.** One-year follow-up was complete for all patients (n = 46, 100%). At the time of the 12-month follow-up, 2 patients had died (4.3%); 1 in the myocardial TTR-negative and 1 in the -positive group (3% vs 33%; P = 0.477). Both the patients had elevated N-terminal pro-B-type natriuretic peptide (NT-proBNP) and low MCF preoperatively, and both died within 3 months of surgery. Both of these patients had amyloid deposits in the aortic valve on histopathological examination but only 1 had TTR deposits.

**DISCUSSION**

In a cohort of 46 elderly patients older than 65 years who were symptomatic with severe AS and underwent SAVR, PYP imaging was performed in 32 patients (70%) that revealed significant radiotracer uptake (Perugini grade II or III) in 3 (9.4%) patients, suggesting myocardial TTR amyloidosis. However, none of the patients showed amyloid deposition in the IVS biopsy specimens. Thirty-three patients (72%) showed Congo red stain positivity in the aortic valves suggestive of isolated valvular amyloid deposition. Of these, 19 patients (58%) had positive staining with TTR-specific antibodies on IHC, suggestive of isolated valvular TTR amyloidosis (Central Illustration).
Although mass spectrometry–based shotgun proteomics would have helped to identify the different types of amyloid in the excised aortic valves, this was performed due to logistical and resource constraints (12).

Projecting these findings to the overall Indian population, the results suggest a huge unmet need for this dual disease’s awareness, detection, and treatment. Extrapolating conservative estimates from our study (n = 3 [9.3%] patients with significant PYP uptake of 32 patients for whom PYP scan was performed) to the 2019 United Nations estimate for Indian population suggests 90 million elderly patients >65 years old (6.6% of 1.36 billion), of whom 4.5 million would have severe AS (13). Nine percent of this amounts to ~400,000 patients expected to have dual AS-ATTR-CA (range: 90,000 to 1,125,000 considering a 95% confidence interval of 2.0% to 25%).

We found isolated valvular amyloidosis in the aortic valves of 33 (72%) patients. This is similar to the amyloid deposition in aortic valves of patients with calcific AS which has been noted previously, ranging from 75% to 100% (14,15). However, the majority of these amyloid deposits had been stained with apolipoprotein A1 (Apo-A1) antibody; this is in contrast to our Congo red-positive patients, where 19 patients (58%) stained positively with TTR antibody. Whereas Kristen et al (14) noted non-amyloidotic Apo-A1 deposition in 74% of severe AS patients, Audet et al (15) did not use anti-TTR antibodies during IHC and found Apo-A1 in all their severe AS patients. This consistency of Congo red detection and variance of amyloid subtyping across studies is likely due to the different methodologies used, as well as the varying racial/demographic profiles. The Apo-A1 deposition in the calcific aortic valves was previously explained by the atherogenic milieu, and is thought to be promoted by dyslipidemia. This is mainly due to local inflammation within the atherosclerotic plaques in the aortic valve which oxidizes the methionine residues of Apo-A1 and transforms them into amyloidogenic proteins (16). These amyloid depositions are believed to cause mineralization of calcified aortic valves by promoting apoptosis of valvular interstitial cells (15). However, there is no benefit of lipid-lowering therapy (statins) on severity or progression of aortic stenosis (17). TTR deposition may be explained by the shear stress caused by the accelerated flow of blood across the stenosed valve predisposing it to amyloidogenesis. Also, TTR deposition was qualitatively observed more prominently adjacent to high calcific burden sites. In contrast to our study, none of these previous 2 studies studying aortic valves looked at amyloid deposition in the IVS (14,15). On the contrary, myocardial amyloidosis, without looking at valvular amyloidosis, has been diagnosed on IVS biopsy by Treibel et al (5).

No previous study has looked at severe AS patients for concomitant ATTR-CA by performing
histopathological examination of cardiac tissue and PYP imaging prospectively and independently. There is only 1 previous study that looked at the presence of ATTR-CA in patients undergoing SAVR; it showed a prevalence of 5.6% (5). Here, detection of amyloid on histopathology was the gold standard, and the same was later correlated with postoperative 3,3-diphosphono-1,2-propanodicarboxylic acid (DPD) scans.

With the advent of TAVR, an increasing number of patients are undergoing AVR without open heart surgery. This is reflected in the recent studies, all of which have attempted to diagnose ATTR-CA by radionuclide scintigrafic imaging tests instead of using IVS biopsy specimens (6,7,18,19). These studies are briefly summarized in Supplemental Table 3 (5-8,18-21). In the absence of tissue histopathological examination, red flags from clinical features, ECG, echo, biomarkers (NT-proBNP and troponin), PYP/DPD scan, and cardiac magnetic resonance imaging should guide amyloid screening in these patients (22).

Radionuclide scintigraphy has become the standard for diagnosis of ATTR-CA as per the guidelines endorsed by ASNC/AHA/ASE (11). Specifically, grade II or III uptake, in the absence of clonal cell expansion, is considered to be highly specific and sensitive (>95%) for ATTR-CA (23). In our cohort, this was seen in 3 patients (9.4% of the 32 patients where PYP imaging was performed), suggesting significant myocardial ATTR-CA. There was no difference in our patients with ATTR-CA (grade II or III uptake) versus...
those without ATTR-CA in terms of age, sex, and grade of symptoms. Variables which differed significantly across this included MCF, deceleration time (dT), GLS, and abnormal GLS. Similar results showing significant diastolic dysfunction (decreased dT) and significantly poor LV function (low MCF) have been shown in previous studies as well (6,18,19,24,25).

Histological alterations in the cardiac valves, including amyloid deposition, are beyond the resolution of scintigraphic scans. PYP scan detects the myocardial-based amyloid deposits, irrespective of the valvular amyloid. PYP grade I uptake might represent a very early phase of myocardial ATTR-CA whose clinical relevance has not yet been proven. However, what has been shown previously is that the prevalence of this subclinical grade I uptake is much higher in patients with severe AS than in age-matched controls without severe AS (21). The same is reflected in our severe AS patients in whom grade I uptake was observed in 6 patients (19%).

The significant PYP uptake noted in 3 patients with negative IVS biopsy specimens suggests substantial myocardial involvement. The lack of amyloid detection in the IVS biopsy samples is likely due to chance factor and/or sampling error. Also, the fact that our study population is at least a decade younger than the other studies (mean age 70 years vs >80 years) may account for a relatively lower burden of amyloid deposition in the myocardium. Importantly, the absence of amyloid deposition in the IVS of our patients must be explored further by inclusion of more patients who test positive on PYP imaging and by subjecting these patients to IVS biopsy intraoperatively during SAVR.

Based on our findings of younger age of the study participants with positive PYP scans but negative IVS biopsy specimens, a plausible hypothesis which merits consideration is that the aortic valve is the site of initial accumulation of TTR deposits, which later progress to involve the myocardium; with progression of age and disease process, more florid deposition of TTR amyloid would occur across the myocardium, which might account for the improved likelihood of amyloid detection on IVS biopsy, as noted by Treibel et al (5) (amyloid detected in 5.6% of patients with mean age of 75 years), or the higher rate of scintigraphic scan-based identification of TTR deposition, as noted by Castano et al (6) (amyloid detected in 16% of patients with mean age of 84 years). However, it is not possible to establish the same from the present study and hence, this remains a hypothetical possibility requiring validation from further research.

At the 1-year follow-up examination, 4.3% patients had died post-SAVR. Although both these patients had Congo red positivity in aortic valves, only 1 of them had significant Perugini grade II or III uptake. Lone AS patients fared better than dual disease (3% vs 33%, respectively). Previous studies have also shown an overall poorer long-term survival despite AVR in severe AS patients who also had myocardial ATTR-CA (5,8). It might be prudent to consider TTR amyloid targeting molecules such as tafamidis in patients with dual AS-ATTR-CA if AVR alone is shown to be less beneficial in this group of patients as compared to those with lone AS. However, benefit from treatment of isolated valvular amyloidosis has not been shown until now. Bearing in mind that >40% of total severe AS patients in this study had isolated valvular TTR amyloidosis and the possibility of valvular amyloidosis progressing to myocardial amyloidosis, it may be worthwhile to consider the impact of agents targeting TTR on the rate of progression of AS severity. Also, early removal of the hemodynamic stress imposed by the stenosed valve (early AVR) may halt/delay the progression of TTR amyloid deposition in severe AS patients with isolated valvular amyloidosis. These therapeutic possibilities are thought-provoking and merit further studies to delineate the therapeutic approaches for both dual AS-ATTR-CA patients and severe AS patients with isolated valvular amyloidosis.

Previous studies, primarily recruiting White populations, have shown the prevalence of concomitant ATTR-CA in severe AS patients to be approximately 15% (Supplemental Table 3). Black Americans are known to have even higher TTR penetrance (9). Before the present study, the distribution of ATTR-CA in an Asian population was unknown. As emphasized above, conservatively estimating a prevalence of dual AS-ATTR-CA as 9.4% (n = 3 of 32) of those with severe AS implies a tremendous unidentified burden of TTR amyloidosis (~400,000 patients with dual AS-ATTR-CA). Although hypothetical as of now, it is intuitive that targeted management of myocardial TTR amyloid will have positive prognostic implications over and above AVR in patients with dual AS-ATTR-CA. Considering this and despite the current cost (>-$100,000 USD annually) and difficult availability of TTR-targeting molecules, it is quintessential to consider their use for these specific dual-disease patients.

**STUDY LIMITATIONS.** ATTR-CA prevalence increases with age. Ours is a younger cohort (mean age: 70 years), compared to other studies (mean age: 80 years). This may have been a potential reason behind the negative IVS biopsy specimens. Studying an older population might have increased the prevalence of myocardial TTR deposits. Amyloid subtyping was
restricted to TTR only. This was performed with IHC using TTR-specific antibodies alone, without use of monospecific antibodies for other amyloid subtypes. Also, laser microdissection and mass spectrosopic analysis were not performed, which would have helped to identify the other different types of amyloid in the excised aortic valves. Being both the first study to look at ATTR-CA in resource-constrained settings and a pilot study, the sample size and follow-up duration is too small to justifiably draw inferences regarding the predictors of mortality post-SAVR in this group of patients. Also, no adjustment for multiple testing was performed because of the limited sample size, and results should be interpreted accordingly.

CONCLUSIONS

The present study shows that concomitant existence of myocardial ATTR-CA in severe AS is not uncommon, as detected by 99m-technetium pyrophosphate imaging (9%). Noninvasive markers of this dual disease include significantly low myocardial contraction fraction, deceleration time, and GLS. Amyloid deposition in the aortic valves of patients with severe AS is much more common, as detected by histopathological examination (72%), significance of which remains unknown. The majority of the patients with amyloid in aortic valves showed deposition of TTR as detected by immunohistochemistry (58%). Future larger studies are warranted to actively investigate this concept and its clinical significance.

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APPENDIX For supplemental tables and a figure, please see the online version of this paper.