

# Light-chain and transthyretin cardiac amyloidosis in severe aortic stenosis: prevalence, screening possibilities, and outcome

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## Aims

Concomitant cardiac amyloidosis (CA) in severe aortic stenosis (AS) is difficult to recognize, since both conditions are associated with concentric left ventricular thickening. We aimed to assess type, frequency, screening parameters, and prognostic implications of CA in AS.

## Methods and results

A total of 191 consecutive AS patients ( $81.2 \pm 7.4$  years; 50.3% female) scheduled for transcatheter aortic valve replacement (TAVR) were prospectively enrolled. Overall, 81.7% underwent complete assessment including echocardiography with strain analysis, electrocardiography (ECG), cardiac magnetic resonance imaging (CMR), <sup>99m</sup>Tc-DPD scintigraphy, serum and urine free light chain measurement, and myocardial biopsy in immunoglobulin light chain (AL)-CA. Voltage/mass ratio (VMR; Sokolow–Lyon index on ECG/left ventricular mass index) and stroke volume index (SVi) were tested as screening parameters. Receiver operating characteristic curve, binary logistic regression, and Kaplan–Meier curve analyses were performed. CA was found in 8.4% of patients ( $n = 16$ ); 15 had transthyretin (TTR)-CA and one AL-CA. While global longitudinal strain by echo did not reliably differentiate AS from CA-AS [area under the curve (AUC) 0.643], VMR as well as SVi showed good discriminative power (AUC 0.770 and 0.773, respectively), which was comparable to extracellular volume by CMR (AUC 0.756). Also, VMR and SVi were independently associated with CA by multivariate logistic regression analysis ( $P = 0.016$  and  $P = 0.027$ , respectively). CA did not significantly affect survival  $15.3 \pm 7.9$  months after TAVR ( $P = 0.972$ ).

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**Conclusion**

Both TTR- and AL-CA can accompany severe AS. Parameters solely based on ECG and echocardiography allow for the identification of the majority of CA-AS. In the present cohort, CA did not significantly worsen prognosis 15.3 months after TAVR.

**Keywords**

Aortic stenosis • Cardiac amyloidosis • Transcatheter aortic valve replacement • Screening • Prognosis

**Introduction**

Degenerative aortic stenosis (AS) and cardiac amyloidosis (CA) are both frequent and serious conditions in the elderly, causing considerable treatment expenditure.<sup>1,2</sup> CA is caused by myocardial deposition of amyloid fibrils. The two predominant amyloid proteins found in the heart are transthyretin (TTR) and immunoglobulin light chain (AL).<sup>3</sup> Expansion of the extracellular space resulting from amyloid deposition leads to myocardial stiffening and restrictive filling of the left ventricle. In addition, AL amyloid may exhibit direct toxic effects on myocardial cells impairing systolic left ventricular (LV) function.<sup>4–6</sup> Affected patients develop severe heart failure and face a dismal prognosis.<sup>7</sup> Formerly believed to be a rare condition, the use of modern diagnostic modalities such as cardiac magnetic resonance (CMR) imaging and <sup>99m</sup>Tc-labeled 3,3-diphosphono-1,2-propanodicarboxylic acid (<sup>99m</sup>Tc-DPD) bone scintigraphy have recently led to a considerable increase in the detection of CA.<sup>8,9</sup>

Latest studies have drawn attention to coexisting CA in patients with degenerative AS.<sup>8–12</sup> In these patients, significant myocardial thickening is naturally attributed to long-standing pressure overload and not recognized as a potential sign for the presence of a storage disease. Coexisting CA in patients with AS has been reported to be associated with worse outcome.<sup>8</sup> Furthermore, management of these patients is a matter of discussion since they may benefit less from surgical (SAVR) or transcatheter aortic valve replacement (TAVR).<sup>13</sup> Present data on CA in AS patients largely rely on CMR, transthoracic echocardiography, and bone scintigraphy as diagnostic tools. <sup>99m</sup>Tc-DPD bone scintigraphy allows for non-invasive detection of TTR-CA with high diagnostic sensitivity and specificity.<sup>14</sup> However, in AL-CA <sup>99m</sup>Tc-DPD scintigraphy may be unremarkable and assessment of serum and urine samples as well as myocardial biopsy may be required to establish the exact diagnosis.<sup>15</sup> Previous studies on CA in AS have not described any cases of AL-CA.<sup>9,10</sup>

The presence of TTR-CA has been linked to worse outcomes following SAVR.<sup>8,10</sup> Patients in these former studies were significantly younger as compared to a classic TAVR cohort. Therefore, at this stage it is unclear whether concomitant CA also holds worse survival implications for patients undergoing TAVR.

As the prevalence of AS is high, comprehensive systematic screening for concomitant CA is difficult, if not sometimes impossible. Many cardiovascular centres lack nuclear imaging facilities and will not always have oncologists available to discuss serum/urine sample reports. CMR including contrast studies is also not applicable in every single AS patient. Thus, screening possibilities solely on

the basis of echocardiography and electrocardiogram (ECG) would be an important step forward.

The present study was designed to systematically assess the prevalence of both TTR- and AL-CA in degenerative AS scheduled for TAVR, to investigate the impact of CA on survival following TAVR, and to evaluate parameters based on echocardiography and ECG that may suggest the presence of CA.

**Methods****Study population**

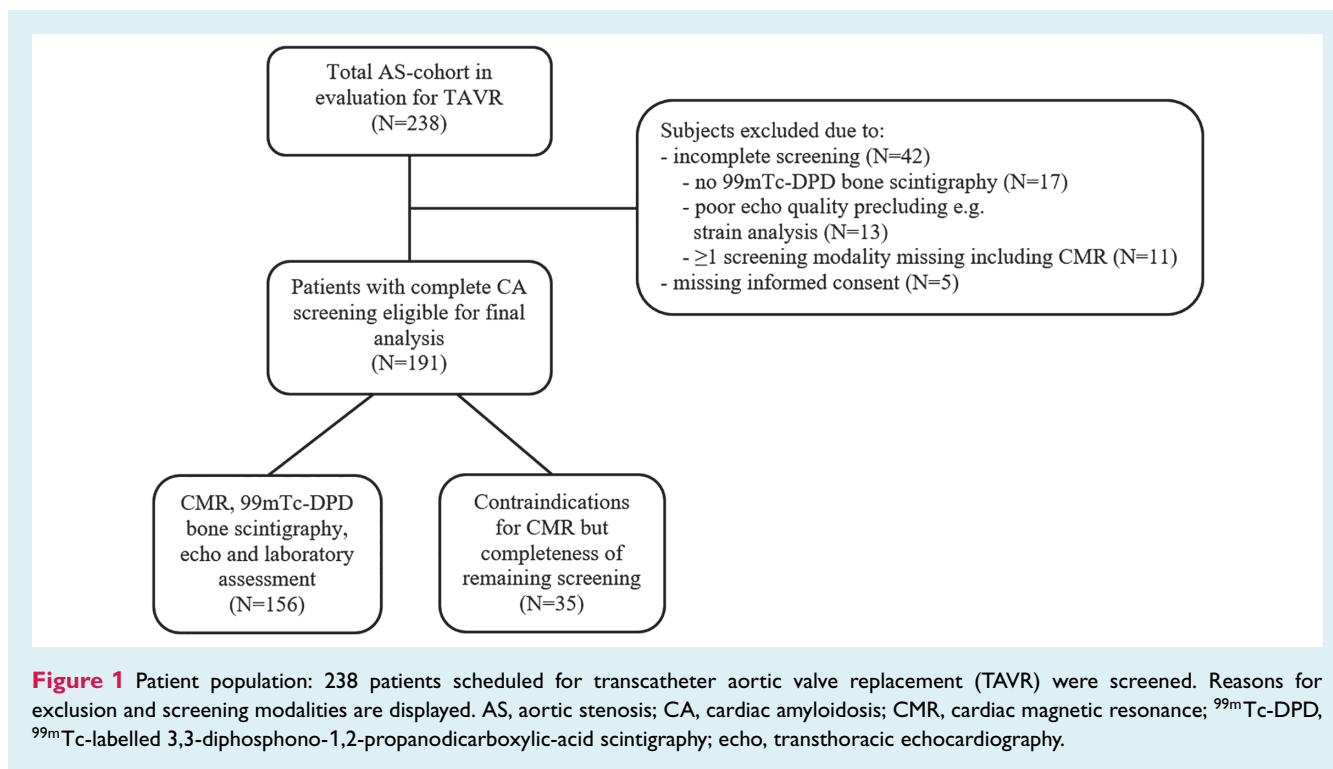
Between October 2017 and January 2019, we prospectively enrolled consecutive adult patients with severe degenerative AS scheduled for TAVR at the Vienna General Hospital, a university-affiliated tertiary centre. Eligibility and decision for TAVR were determined by a multidisciplinary Heart Team. Patients underwent clinical and laboratory assessment, ECG, transthoracic echocardiography with strain rate analysis, CMR, and <sup>99m</sup>Tc-DPD bone scintigraphy prior to TAVR. Overall, 81.7% ( $n = 156/191$ ) underwent all diagnostic modalities, 18.3% ( $n = 35/191$ ) had contraindications precluding CMR (*Figure 1*). In case of suspicion of AL-CA (presence of monoclonal protein on serum or urine immunofixation ± abnormal free light chain ratio on serum analysis ± abnormal urine protein/creatinine or albumin/creatinine ratio AND abnormal findings on CMR or bone scintigraphy,  $n = 2$ ), right ventricular myocardial biopsy was performed. Patients were followed by echocardiography, ECG, and clinical and laboratory assessment. All-cause death and cardiovascular hospitalization were selected as primary and secondary study endpoints, respectively. All patients provided written informed consent. The study was approved by the Ethics Committee of the Medical University of Vienna (EK no. 2218/2016).

**Diagnosis of cardiac amyloidosis**

Transthyretin CA was defined as the presence of cardiac tracer uptake Perugini grade  $\geq 2$  on bone scintigraphy in patients with unremarkable serum and urine free light chain assessment.<sup>14</sup> AL-CA was diagnosed if endomyocardial or extracardiac biopsy specimen and consecutive immunohistochemical analysis revealed deposition of light chains, combined with elevated serum or urine levels of the corresponding monoclonal light chain. In AL cases with extracardiac biopsies only, cardiac imaging indicating cardiac involvement was required for establishing the diagnosis.

**Clinical, laboratory and electrocardiographic assessment**

For the detection of pathological light chains underlying AL-CA, laboratory testing included serum electrophoresis, immunoglobulin and free



light chain quantification and immunofixation. Urine analysis consisted of electrophoresis, immunofixation, protein/creatinine ratio and albumin/creatinine ratio. Rare causes of CA were ruled out via quantification of serum amyloid A and  $\beta$ 2-microglobulin. Additionally, N-terminal pro-brain natriuretic peptide (NT-proBNP) serum levels were determined in all patients.

Electrocardiograms were recorded according to current recommendations.<sup>16</sup> Voltage/mass ratio (VMR) was determined in patients without bundle branch block and paced rhythm by dividing the Sokolow–Lyon index by the LV mass index on echocardiography. The Sokolow–Lyon index was calculated as the sum of precordial voltage [S-wave in lead V<sub>1</sub> plus R-wave in lead V<sub>5</sub> or V<sub>6</sub> (SV<sub>1</sub> + RV<sub>5</sub> or V<sub>6</sub>)].

## Echocardiographic assessment

Transthoracic echocardiography was performed by board certified cardiologists with echocardiography systems equipped with 3.5 MHz transducers (Vivid E95, Vivid E9, Vivid S70; General Electric Healthcare). LV ejection fraction was calculated with the biplane Simpson's method and valvular stenosis and regurgitation severity were quantified according to the respective guidelines.<sup>17</sup> Strain analysis was performed in the 4-, 3-, and 2-chamber apical views. Regional longitudinal strain (LS) was determined in 17 segments of the left ventricle.<sup>18</sup> Global LS was calculated as the average LS of these 17 segments. Relative apical LS was calculated as average apical LS/(average basal LS + average mid LS). LV mass was determined by anatomical M-mode as previously described.<sup>19</sup> LV stroke volume was derived from the LV outflow tract pulsed-wave Doppler signals and indexed to body surface area to receive stroke volume index (SV<sub>i</sub>). Myocardial contraction fraction was calculated as the ratio of stroke volume to myocardial volume, with the latter being calculated from linear dimensions in the parasternal long-axis view.

## Cardiac magnetic resonance imaging

Cardiac magnetic resonance examinations were performed on a 1.5 T scanner (MAGNETOM Avanto; Siemens Healthcare GmbH, Erlangen, Germany), following standard protocols that included late gadolinium enhancement (LGE) imaging (0.1 mmol/kg gadobutrol; Gadovist, Bayer Vital GmbH, Leverkusen, Germany) if estimated glomerular filtration rate was  $\geq 30$  mL/min/1.73 m<sup>2</sup>.<sup>20</sup> At the time of insertion of the intravenous cannula, blood was drawn for haematocrit and serum creatinine measurement. For analysis of LGE images, two independent reviewers judged whether a typical pattern for CA was present or not.<sup>21</sup> Electrocardiographically triggered modified look-locker inversion recovery (MOLLI) using a 5(3)3 prototype (5 acquisition heartbeats followed by three recovery heartbeats and further three acquisition heartbeats) was applied for pre-contrast T1 mapping. This method generates an inline, pixel-based T1 map by acquiring a series of images over several heartbeats with shifted T1 times, inline motion correction, and inline calculation of the T1 relaxation curve within one breath hold. T1 sequence parameters were as follows: starting inversion time 120 ms, inversion time increment 80 ms, reconstructed matrix size 256 × 218, and measured matrix size 256 × 144 (phase-encoding resolution 66% and phase-encoding field of view 85%). T1 maps were created both before and 15 min after contrast agent application. For post-contrast T1 mapping, a 4(1)3(1)2 prototype was used. T1 values from a mid-cavity short-axis slice and a mid-cavity 4-chamber view were averaged for assessment of entire LV myocardium. For extracellular volume (ECV) calculation the following formula was used<sup>22</sup>:

$$\text{MOLLI} - \text{ECV} = 1 - \text{haematocrit} \times \left( \frac{\frac{1}{T1_{\text{myopost}}} - \left( \frac{1}{T1_{\text{myopre}}} \right)}{\left( \frac{1}{T1_{\text{bloodpost}}} - \left( \frac{1}{T1_{\text{bloodpre}}} \right) \right)} \right)$$

T1 myo pre/T1 blood pre indicates myocardial/blood native T1 times and T1 myo post/T1 blood post indicates T1 times of

myocardium/blood 15 min after gadobutrol application. The local reference range for normal MOLLI-ECV values is  $25.4 \pm 2.7\%$ , derived from 36 healthy sex-matched controls.<sup>23</sup> ECV quantification applying the MOLLI sequence has been shown to correlate strongly with histological fibrosis.<sup>24</sup>

### <sup>99m</sup>Tc-DPD bone scintigraphy

All patients were scanned using either a General Electric (GE) Infinia Hawkeye 4 or GE Discovery 670 hybrid gamma camera 3 h after intravenous administration of 700 MBq of <sup>99m</sup>Tc-DPD. Whole body images were acquired at a scan speed of 10 cm/min using low-energy high-resolution collimators.<sup>25</sup> The expected radiation dose from the entire procedure was 4 mSv per patient. Intensity of myocardial uptake on planar <sup>99m</sup>Tc-DPD bone scintigraphy was categorized as 0–3 according to the Perugini grading system.<sup>26</sup> This visual categorization can be summed up as follows: grade 0, no cardiac uptake and normal bone uptake; grade 1, cardiac uptake which is less intense than the bone signal; grade 2, cardiac uptake with intensity similar or greater than bone signal; and grade 3, cardiac uptake with much attenuated or absent bone signal. The heart-to-contralateral ratio was determined by drawing a region of interest over the heart, copying it and mirroring it over the contralateral chest.<sup>27</sup>

### Myocardial biopsy and genetic testing

Biopsies were harvested from the left ventricle using a 6 F biptome. Histological analysis was performed by Congo red staining on 6 µm formalin-fixed and paraffin-embedded sections and viewed in brightfield and cross-polarized light. When amyloid was confirmed by displaying apple green birefringence under cross-polars, immunohistochemical analysis (AmY-kit amyloid antibodies, Martinsried, Germany) was performed to determine the amyloid subtype. For genetic testing in patients with TTR-CA, the complete coding regions of the TTR gene were amplified by polymerase chain reaction assay. Amplified DNA fragments were directly sequenced using an ABI 3130xl Genetic Analyzer (Applied Biosystems).

### Statistical methods

Continuous data are expressed as mean  $\pm$  standard deviation (SD), or as median with corresponding interquartile range (IQR) and categorical variables are presented as percentages or total numbers. Differences between groups were analysed with the Wilcoxon rank sum test. Chi-square tests or Fisher exact tests were used for categorical variables as appropriate. To analyse ECV expansion with respect to different health conditions, MOLLI-ECV was presented as mean  $\pm$  SD and compared using box-plots. To estimate the discriminative power of parameters in the distinction of AS and CA-AS, areas under the corresponding receiver operating characteristic (ROC) curves with respective 95% confidence intervals (CI) were established and compared. Uni- and multivariate binary logistic regression analysis were applied to evaluate the association of parameters with the presence of CA. For each group (baseline clinical, echo-, and electrocardiographic values) multivariate analysis was performed using a stepwise forward selection with the cut-off *P*-value to enter the multivariate model being  $\leq 0.05$  (online supplementary Table S2). The parameter remaining in the model was then selected to enter the final multivariate non-stepwise analysis alongside with SVi and VMR, respectively. To allow better comparison between continuous parameters

within the multivariate model, scaled hazard ratios (Z-scores) were created by subtracting the mean from individual values and dividing them by the respective SD. Kaplan–Meier curves were used to evaluate the prognostic significance of CA in patients after TAVR. A *P*-value  $\leq 0.05$  was considered statistically significant. All statistical analyses were computed using SPSS 24 (IBM SPSS, Chicago, IL, USA).

## Results

### Patient population

A total of 238 consecutive patients scheduled for TAVR were included (online supplementary Table S1). Contraindications for CMR were present in 46 subjects, who were still included in the final analysis if the remaining screening was complete ( $n = 35$ ). Whenever AL-CA was suspected, patients underwent additional bone marrow and myocardial biopsy. The patient population eligible for final analysis is displayed in Figure 1.

In total, 16 (8.4%) CA cases were observed. TTR-CA was diagnosed in 15 subjects, one patient suffered from AL-CA. Genetic analysis confirmed wild-type TTR amyloidosis in all 15 cases, the patient with AL-amyloidosis had monoclonal IgG gammopathy with excess production of  $\kappa$ -light chains.

### Clinical, laboratory and electrocardiographic assessment

Detailed baseline characteristics of patients stratified according to presence of coexisting CA are displayed in Tables 1 and 2. Patients with CA were older [84.0 years (IQR 82.0–89.0) vs. 82.0 years (77.0–85.2),  $P = 0.024$ ], had lower systolic blood pressure levels [119 mmHg (108–130) vs. 132 mmHg (120–145);  $P = 0.010$ ], and a higher prevalence of cardiac pacemakers pre-TAVR (31.2% vs. 11.7%;  $P = 0.040$ ) as compared to AS subjects without concomitant CA. Regarding the cardiovascular risk profile, no significant differences were observed between groups. NT-proBNP [3634 ng/dL (1241–6323) vs. 1839 ng/dL (727–5664)] as well as troponin T serum levels [47.0 ng/L (24.0–72.0) vs. 28.0 ng/L (20.0–48.7)] did not differ significantly between CA and non-CA ( $P$  for both  $>0.05$ ).

On ECG, patients with coexisting CA displayed a significantly lower Sokolow–Lyon index [1.7 mV (1.1–2.3) vs. 2.2 mV (1.6–2.8);  $P = 0.028$ ].

### Transthoracic echocardiography

Echocardiographic details are shown in Table 2. CA patients displayed lower transvalvular aortic mean [35.0 mmHg (26.0–48.5) vs. 47.0 mmHg (40.0–56.0);  $P = 0.004$ ] and peak pressure gradients [60.0 mmHg (43.0–73.5) vs. 77.0 mmHg (67.0–92.0);  $P = 0.001$ ]. Moreover, patients with CA-AS had higher LV mass indices [159.0 g/m<sup>2</sup> (132.0–185.5) vs. 135.0 g/m<sup>2</sup> (111.8–162.3);  $P = 0.016$ ] and a lower myocardial contraction fraction [15.1% (9.8–19.1) vs. 21.9% (17.1–27.2);  $P = 0.001$ ]. On the contrary, LV ejection fraction was similar [62.0% (44.0–70.0) vs. 62.0%

**Table 1** Baseline clinical parameters

	All patients (n = 191)	No CA (n = 175, 91.6%)	CA (n = 16, 8.4%)	P-value
Age, years	82.0 (78.0–86.0)	82.0 (77.0–85.2)	84.0 (81.0–89.0)	<b>0.024</b>
Male sex	49.7	48.3	62.5	0.196
BMI, kg/m <sup>2</sup>	26.1 (23.4–29.7)	26.1 (23.5–29.9)	25.7 (22.9–29.5)	0.405
Arterial hypertension	87.3	87.2	87.5	0.904
Systolic BP, mmHg	130 (118–145)	132 (120–145)	119 (108–130)	<b>0.010</b>
Diastolic BP, mmHg	67 (58–78)	67 (58–78)	67 (60–78)	0.908
Pre-interventional PM	13.1	11.7	31.2	<b>0.040</b>
Diabetes	26.2	25.9	31.2	0.751
Atrial fibrillation	37.7	35.6	56.3	0.060
Hyperlipidaemia	66.0	65.5	68.8	0.673
CAD	48.7	50.0	37.5	0.247
PAD	12.2	13.5	0.0	0.107
Severe symptomatic AS stage				<b>0.026</b>
High gradient	72.3	75.4	43.8	
LF-LG + EF <50%	9.4	9.2	12.5	
LF-LG + EF ≥50%	18.2	15.5	43.8	
NYHA class ≥III	62.4	62.4	62.5	0.995
CCS class ≥III	7.2	7.3	6.3	0.875
Syncope	10.9	12.0	0.0	0.142
Troponin T, ng/L	29 (20–50)	28 (20–49)	47 (24–72)	0.107
NT-proBNP, ng/dL	1917 (783–5893)	1839 (727–5664)	3634 (1241–6323)	0.316
eGFR, mL/min/1.73 m <sup>2</sup>	55.1 (42.7–73.2)	55.4 (42.9–73.0)	55.1 (42.3–80.2)	0.831
EuroSCORE II, %	4.2 (3.9–4.7)	4.2 (3.9–4.7)	4.5 (4.0–4.6)	0.419
STS score, %	3.7 (2.5–5.3)	3.5 (2.5–5.1)	4.7(3.5–5.7)	<b>0.037</b>

Values are given as %, or median (interquartile range).

AS, aortic stenosis; BMI, body mass index; BP, blood pressure; CA, cardiac amyloidosis; CAD, coronary artery disease; CCS, Canadian Cardiovascular Society; EF, ejection fraction; eGFR, estimated glomerular filtration rate; LF-LG, low-flow low-gradient; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; PAD, peripheral artery disease; PM, pacemaker; STS, Society of Thoracic Surgeons.

(54.0–70.0);  $P = 0.576$ ]. This is also reflected by a higher percentage of paradoxical low-flow low-gradient AS among CA-AS (43.8% vs. 15.5%). While speckle-tracking analysis revealed more severe basal and midventricular LS impairment in CA-AS ( $P$  for both  $<0.05$ ), there were no significant differences between groups with respect to global [−13.8% (−16.6; −10.2) vs. −16.9% (−19.6; −12.3)] and relative apical LS [0.92 (0.81–1.20) vs. 0.81 (0.73–0.96);  $P > 0.05$ ]. In addition, CA patients more often suffered from moderate or severe tricuspid regurgitation (62.5% vs. 29.4;  $P = 0.017$ ).

## Cardiac magnetic resonance imaging

Detailed CMR data are shown in Table 2. Patients with and without CA did not display significant differences with respect to left and right heart dimensions ( $P$ -values for all chambers  $>0.05$ ) and LV mass indices [93.9 g/m<sup>2</sup> (61.3–100.5) vs. 79.4 g/m<sup>2</sup> (63.3–90.2);  $P = 0.163$ ]. On the contrary, ECV values were significantly higher among CA-AS [(30.3% (28.1–33.5) vs. 26.7% (24.6–29.0);  $P = 0.003$ ], even though there was a considerable overlap of isolated AS and CA-AS (online supplementary Figure S1). Importantly, among the 11 CA patients who underwent CMR, characteristic transmural LGE pattern was only present in four cases (all TTR-CA). In the remaining seven CA patients, ECV values

were not remarkably elevated and typical LGE pattern was absent (online supplementary Table S3).

## <sup>99m</sup>Tc-DPD bone scintigraphy

Among TTR-CA patients ( $n = 15$ ), <sup>99m</sup>Tc-DPD bone scintigraphy revealed grade 1 [ $n = 1$  who underwent confirmatory endomyocardial biopsy (EMB)], grade 2 ( $n = 10$ ) and grade 3 ( $n = 4$ ) cardiac uptake, whereas no cardiac uptake was seen in the patient with isolated AL-CA. The TTR patient with grade 1 uptake underwent additional cardiac biopsy, which eventually confirmed the diagnosis. Conversely, grade 1 uptake was seen in another five subjects with unremarkable free light chain assessment, who all declined to undergo cardiac biopsy. Therefore, according to consensus criteria,<sup>14</sup> the diagnosis of TTR-CA could not be confirmed in these patients. By quantitative assessment of <sup>99m</sup>Tc-DPD myocardial uptake, CA patients showed an increased heart-to-contralateral ratio [1.79 (1.59–2.31) vs. 1.05 (0.95–1.15);  $P < 0.001$ ].

## Voltage/mass ratio

The VMR was significantly lower in CA patients [ $0.9 \times 10^{-2}$  mV/g/m<sup>2</sup> (0.6–1.6) vs.  $1.6 \times 10^{-2}$  mV/g/m<sup>2</sup> (1.1–2.3);  $P = 0.001$ ]. By ROC analysis, VMR demonstrated good

**Table 2** Baseline parameters of diagnostic modalities

	All patients (n = 191)	No CA (n = 175, 91.6%)	CA (n = 16, 8.4%)	P-value
<b>Echocardiographic parameters</b>				
LA diameter, mm	61.0 (55.8–67.3)	61.0 (55.0–68.0)	64.0 (60.0–67.0)	0.215
RA diameter, mm	57.0 (51.0–64.0)	56.0 (51.0–63.0)	63.0 (54.0–70.0)	<b>0.029</b>
LVEDD, mm	44.0 (39.0–48.0)	44.0 (39.3–48.0)	41.5 (37.0–48.8)	0.246
RVEDD, mm	33.0 (29.3–38.0)	33.0 (29.5–37.0)	36.0 (28.0–43.0)	0.144
IVS, mm	15.0 (14.0–17.0)	15.0 (14.0–17.0)	15.5 (13.3–19.8)	0.183
AVA, cm <sup>2</sup>	0.6 (0.5–0.8)	0.6 (0.5–0.8)	0.6 (0.5–0.9)	0.669
AV PPG, mmHg	73.0 (64.0–92.0)	77.0 (67.0–92.0)	60.0 (43.0–73.5)	<b>0.001</b>
AV MPG, mmHg	46.0 (38.0–55.5)	47.5 (40.0–56.0)	35.0 (26.0–48.5)	<b>0.004</b>
MR grade ≥ II	31.2	29.1	56.3	0.065
TR grade ≥ II	32.1	29.4	62.5	<b>0.017</b>
LVEF, %	62.0 (52.5–70.0)	62.0 (54.0–70.0)	62.0 (44.0–70.0)	0.576
LVEDV, mL	79.5 (62.0–108.5)	81.0 (62.5–111.0)	72.0 (59.0–89.5)	0.117
LVEDVi, mL/m <sup>2</sup>	44.3 (33.7–57.3)	44.6 (34.9–58.9)	37.5 (31.5–48.5)	0.097
LVESV, mL	30.0 (20.8–49.0)	30.0 (20.5–49.0)	27.0 (20.0–45.5)	0.567
SVi, mL/m <sup>2</sup>	42.5 (27.5–61.6)	46.6 (29.0–63.7)	27.4 (22.3–33.7)	<b>&lt;0.001</b>
Peak TR velocity, m/s	3.0 (2.7–3.5)	3.0 (2.7–3.4)	3.3 (2.6–4.1)	0.357
sPAP, mmHg	46.0 (39.0–59.0)	46.0 (38.5–57.5)	53.0 (42.0–77.8)	0.173
LV mass index, g/m <sup>2</sup>	136.0 (115.0–164.0)	135.0 (111.8–162.3)	159.0 (132.0–185.5)	<b>0.016</b>
Deceleration time, ms	206 (159–268)	212 (160–275)	199 (145–232)	0.161
MCF, %	21.0 (16.0–26.4)	21.9 (17.1–27.2)	15.1 (9.75–19.1)	<b>0.001</b>
Apical LS, %	–22.0 (–27.5; –16.8)	–22.2 (–28.2; –16.8)	–20.2 (–25.2; –16.0)	0.253
Midventricular LS, %	–15.0 (–18.0; –10.7)	–15.6 (–18.0; –11.1)	–12.8 (–13.8; –9.5)	<b>0.043</b>
Basal LS, %	–10.8 (–13.5; –8.5)	–11.2 (–14.1; –8.8)	–9.7 (–10.8; –5.3)	<b>0.040</b>
GLS, %	–16.6 (–19.5; –11.3)	–16.9 (–19.6; –12.3)	–13.8 (–16.6; –10.2)	0.072
Apical/(mid + basal)	0.83 (0.73–0.98)	0.81 (0.73–0.96)	0.92 (0.81–1.20)	0.061
Cutoff ≥ 1.0 <sup>a</sup>	23.8	21.4	43.8	0.071
Apical/basal	1.89 (1.61–2.41)	1.87 (1.59–2.38)	2.02 (1.58–3.25)	0.378
(Apical+mid)/basal	3.22 (2.76–3.83)	3.20 (2.76–3.78)	3.35 (2.83–5.18)	0.400
<b>Cardiac magnetic resonance parameters</b>				
LVEF, %	61.0 (44.5–72.0)	61.5 (44.0–72.0)	55.0 (47.0–66.0)	0.521
LVEDV, mL	136.0 (106.0–169.5)	134.5 (106.0–169.0)	157.0 (99.0–184.0)	0.374
LVESV, mL	52.0 (32.0–93.5)	51.0 (30.0–94.3)	62.0 (44.0–85.0)	0.362
LV CO, L/min	5.4 (4.2–6.4)	5.4 (4.2–6.4)	5.1 (4.6–6.8)	0.936
RVEF, %	55.0 (43.8–63.0)	55.0 (44.6–63.0)	48.0 (36.0–63.0)	0.271
RVEDV, mL	127.5 (108.0–166.0)	126.0 (107.0–163.0)	163.0 (125.0–203.0)	0.064
RVESV, mL	60.0 (42.8–82.3)	59.0 (42.0–81.0)	82.0 (50.0–123.0)	0.089
RV CO, L/min	4.6 (3.8–5.7)	4.6 (3.7–5.7)	5.0 (4.8–5.8)	0.252
LA area, cm <sup>2</sup>	29.0 (26.0–35.0)	29.0 (26.0–35.0)	31.0 (23.0–34.0)	0.854
RA area, cm <sup>2</sup>	25.0 (20.0–30.0)	25.0 (20.0–30.0)	26.0 (23.0–35.0)	0.428
LV mass, g	147.0 (119.0–182.0)	145.0 (118.0–177.0)	175.0 (130.0–189.0)	0.151
LV mass index, g/m <sup>2</sup>	80.0 (63.2–94.6)	79.4 (63.3–90.2)	93.9 (61.3–100.5)	0.163
Native T1 LV, ms	1036 (1009–1066)	1033 (1008–1063)	1051 (1013–1080)	0.196
MOLLI-ECV LV, %	27.0 (24.9–29.4)	26.7 (24.6–29.0)	30.3 (28.1–33.5)	<b>0.003</b>
<b>ECG parameters</b>				
Heart rate, bpm	70 (62–78)	70 (62–78)	71 (66–77)	0.741
Sokolow–Lyon index, mV	2.1 (1.6–2.8)	2.2 (1.6–2.8)	1.7 (1.1–2.3)	<b>0.028</b>
VMR, mV/g/m <sup>2</sup> × 10 <sup>–2</sup>	1.5 (1.0–2.2)	1.6 (1.1–2.3)	0.9 (0.6–1.6)	<b>0.001</b>
Low voltage limb	6.3	6.4	5.9	0.938
QRS duration, ms	98 (86–118)	96 (84–116)	111 (90–132)	0.086
LBBB	6.6	7.3	0.0	0.262
RBBB	12.0	11.3	18.8	0.386
LAFB	16.3	14.7	31.3	0.088
1st degree AV block	20.0	18.6	33.3	0.225

**Table 2 (Continued)**

	All patients (n = 191)	No CA (n = 175, 91.6%)	CA (n = 16, 8.4%)	P-value
<b>Bone scintigraphy parameters</b>				
Perugini grading scale				<0.001
0	89.0	97.1	6.3	
1	3.1	2.9	6.3 <sup>b</sup>	
2	5.8	0.0	62.5	
3	2.1	0.0	25.0	
H/L ratio	1.07 (0.96–1.19)	1.05 (0.95–1.15)	1.79 (1.59–2.31)	<0.001

Values are given as %, or median (interquartile range).

AV, aortic valve/atrioventricular; AVA, aortic valve area; CO, cardiac output; GLS, global longitudinal strain; H/L ratio, heart-to-contralateral ratio; IVS, interventricular septum; LA, left atrial; LAFB, left anterior fascicular block; LBBB, left bundle branch block; LS, longitudinal strain; LV, left ventricular; LVEDD, left ventricular end-diastolic diameter; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; MCF, myocardial contraction fraction; MOLLI-ECV, extracellular volume fraction as determined by modified look-locker inversion recovery; MPG, mean pressure gradient; MR, mitral regurgitation; PPG, peak pressure gradient; RA, right atrial; RBBB, right bundle branch block; RV, right ventricular; RVEDD, right ventricular end-diastolic diameter; RVEDV, right ventricular end-diastolic volume; RVEF, right ventricular ejection fraction; sPAP, systolic pulmonary artery pressure; SVi, stroke volume index; T1, T1 relaxation time; TR, tricuspid regurgitation; VMR, voltage/mass ratio.

<sup>a</sup>Cutoff value for relative apical strain selected according to study by Phelan et al.<sup>28</sup>

<sup>b</sup>Transthyretin cardiac amyloidosis in this patient with grade 1 uptake was confirmed by endomyocardial biopsy.

**Table 3 Uni- and multivariate binary logistic regression analysis assessing the association of parameters with the presence of cardiac amyloidosis**

	Univariate OR (95% CI)	P-value	Multivariate OR (95% CI)	P-value
<b>Model 1</b>				
Age (per year increase)	1.108 (1.012–1.213)	0.027	4.686 (1.467–14.975)	0.009
MCF	0.353 (0.181–0.691)	0.002	0.657 (0.303–1.422)	0.286
Sokolow–Lyon index	0.481 (0.232–0.998)	0.049	2.740 (0.690–10.886)	0.152
VMR	0.230 (0.084–0.626)	0.004	0.063 (0.007–0.593)	<b>0.016</b>
<b>Model 2</b>				
Age (per year increase)	1.108 (1.012–1.213)	0.027	2.369 (0.906–6.197)	0.079
MCF	0.353 (0.181–0.691)	0.002	0.588 (0.271–1.275)	0.179
Sokolow–Lyon index	0.481 (0.232–0.998)	0.049	0.768 (0.347–1.703)	0.516
SVi	0.209 (0.078–0.559)	0.002	0.296 (0.101–0.869)	<b>0.027</b>

CI, confidence interval; MCF, myocardial contraction fraction; OR, odds ratio; SVi, stroke volume index; VMR, voltage/mass ratio.

discriminative power for the detection of CA-AS [area under the curve (AUC) 0.770, 95% CI 0.641–0.900;  $P = 0.001$ ] (Figure 2). In addition, VMR was associated with CA by univariate linear regression analysis [odds ratio (OR) 0.230, 95% CI 0.084–0.626;  $P = 0.004$ ]. After multivariate adjustment, VMR remained independently linked to the presence of CA (OR 0.063, 95% CI 0.007–0.593;  $P = 0.016$ ) (Table 3).

### Stroke volume index

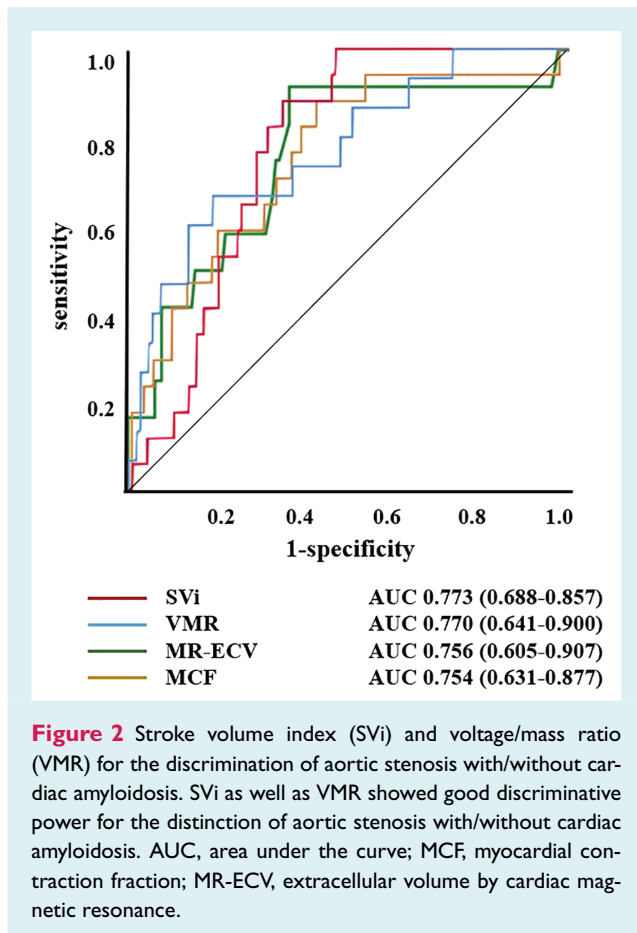
Cardiac amyloidosis patients exhibited a significantly lower SVi [27.4 mL/m<sup>2</sup> (22.3–33.7) vs. 46.6 mL/m<sup>2</sup> (29.0–63.7);  $P < 0.001$ ]. SVi showed good discriminative power by ROC analysis [AUC 0.773, 95% CI 0.688–0.857;  $P < 0.001$  (Figure 2)], and logistic regression analysis revealed a strong association with CA by univariate (OR 0.209, 95% CI 0.078–0.559;  $P = 0.002$ ), and also multivariate analysis (OR 0.296, 95% CI 0.101–0.869;  $P = 0.027$ ) (Table 3).

### Outcome

After a median follow-up of  $15.3 \pm 7.9$  months, 33 patients (3 CA-AS, 30 AS) had died and 22 patients (2 CA-AS, 20 AS) had experienced cardiovascular hospitalizations. One patient without CA died prior to the intervention. Moreover, TAVR was not performed in another five patients (1 CA-AS, 4 AS) due to various reasons, and those subjects were excluded from the outcome analysis. By Kaplan–Meier estimates, the presence of CA was not associated with poorer outcome following TAVR ( $P = 0.972$  and  $P = 0.915$  for primary and secondary endpoints, respectively) (Figure 3).

### Discussion

The present study has four main conclusions: (i) it is the first to describe the presence of AL- as well as TTR-CA among patients with severe AS; (ii) parameters based solely on echocardiography



**Figure 2** Stroke volume index (SVi) and voltage/mass ratio (VMR) for the discrimination of aortic stenosis with/without cardiac amyloidosis. SVi as well as VMR showed good discriminative power for the distinction of aortic stenosis with/without cardiac amyloidosis. AUC, area under the curve; MCF, myocardial contraction fraction; MR-ECV, extracellular volume by cardiac magnetic resonance.

and ECG, that are applicable for CA screening in AS patients, are presented; (iii) the vast majority of CA cases can be detected with  $^{99m}\text{Tc}$ -DPD bone scintigraphy, whereas CMR frequently lacks diagnostic accuracy; and (iv) the presence of CA did not significantly alter prognosis 15.3 months after TAVR.

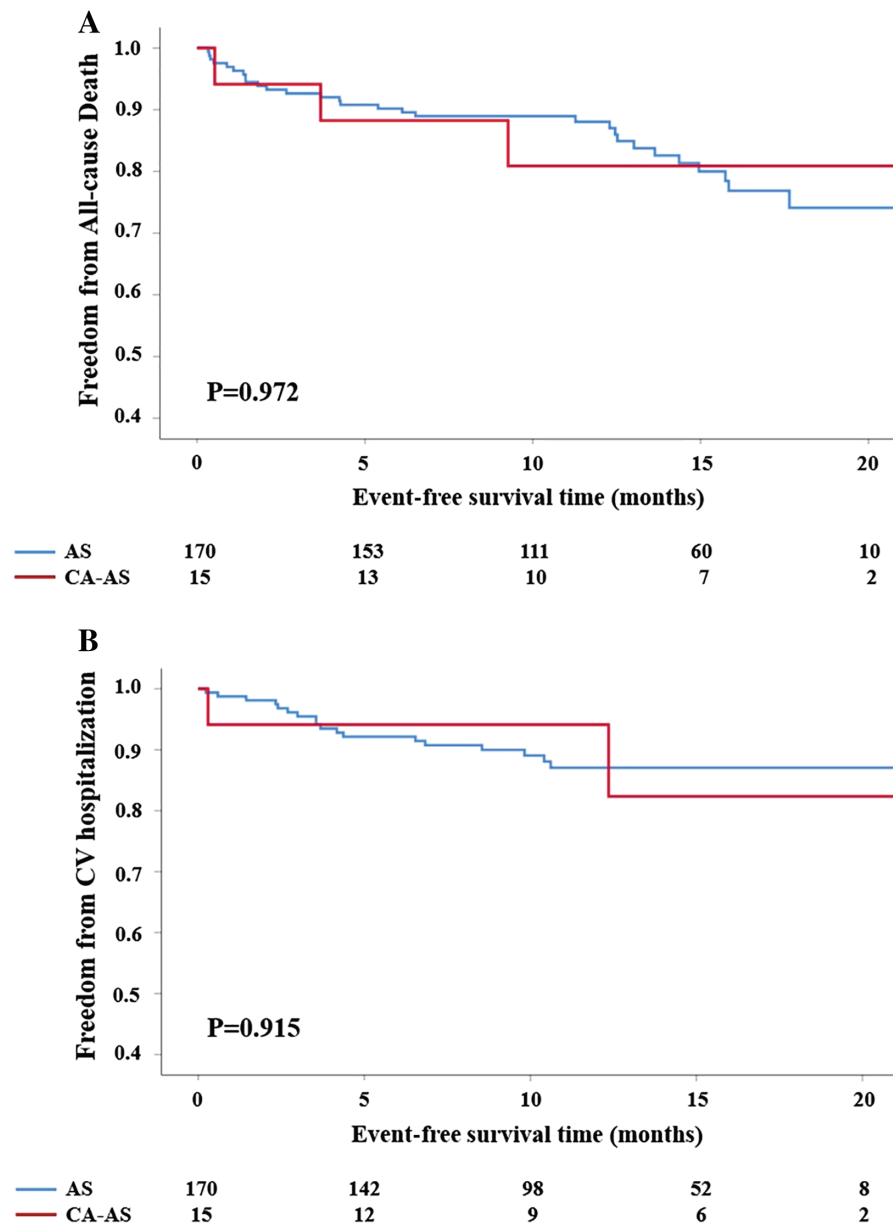
In AS, CA is a difficult diagnosis to make. Due to concentric LV thickening caused by outflow tract obstruction, the distinction between isolated severe AS and AS with coexisting CA is challenging. Recent studies have revealed CA rates ranging from 5% to 16% in patients with severe AS.<sup>8-11</sup> In light of emerging treatment options currently tested in clinical trials (TTR-CA<sup>29</sup> and AL-CA<sup>30</sup>), an exact diagnosis deciphering the underlying pathology to the molecular level is becoming increasingly important. Two previous studies investigated the prevalence of TTR-CA among AS patients scheduled for TAVR using DPD bone scans and identified 16% and 14% of CA, respectively.<sup>9,11</sup> However, in both studies further testing for AL-CA was not pursued despite the presence of monoclonal serum proteins in several patients. Thus, it is possible that subjects in those series suffered from AL-CA or a combination of AL- and TTR-CA but remained undiagnosed. In the work by Treibel *et al.*,<sup>8</sup> 5.2% ( $n = 6$ ) of patients undergoing SAVR were diagnosed with TTR-CA based on intraoperative biopsy. Cavalcante *et al.*,<sup>10</sup> finally, retrospectively analysed CMR scans of 113 AS patients for coexisting CA, and found a prevalence of 8.0%. However, the diagnosis of CA here was solely based on typical LGE

patterns on CMR, and no distinction between TTR- and AL-CA was made.<sup>10</sup>

## Screening for cardiac amyloidosis in aortic stenosis

After systematic screening, a prevalence of 8.4% of concomitant CA was found in the present series, including one case of AL-CA. All previous studies on the prevalence of CA-AS used either CMR or  $^{99m}\text{Tc}$ -DPD bone scintigraphy or myocardial biopsy as single diagnostic modalities. CMR was diagnostic in only 36% of CA-AS in the present study, which confirms the formerly reported low sensitivity of distinctive LGE patterns. In the remainder,  $^{99m}\text{Tc}$ -DPD bone scintigraphy, laboratory assessment, and myocardial biopsy were necessary for CA diagnosis. Interestingly, 37% of CA-AS patients were female, which is in line with previous data on CA among heart failure patients with preserved ejection fraction.<sup>31</sup> However, other investigators reported a significantly lower proportion of women among TTR-CA patients.<sup>14,25,29</sup> The high proportion of females in the present series may be the result of the active screening approach, and may indicate that currently women with CA are potentially missed in clinical routine. Nuclear cardiac imaging with bone seeking radioisotopes allows for non-invasive diagnosis of TTR-CA with high specificity and sensitivity.<sup>14</sup> In contrast, only 30% to 50% of AL-CA patients also display cardiac uptake on DPD bone scans. These patients mostly present with Perugini grade 1 uptake, frequently difficult to detect, also for experienced bone scan readers.<sup>25</sup> In our cohort,  $^{99m}\text{Tc}$ -DPD bone scintigraphy detected the majority of CA-AS cases, but was negative in the patient with isolated AL-CA. Myocardial biopsy, finally, should be performed according to the respective recommendations whenever light chain analysis and consecutive bone marrow biopsy show abnormal results and AL-CA is suspected.<sup>14</sup> However, it is an invasive procedure carrying inherent risks that is, therefore, only recommended in cases with significant probability for AL-CA. In the present cohort of patients with severe AS, myocardial biopsy was performed after TAVR when indicated. Taken altogether, alternative means for the screening for CA-AS based on widely available and low-cost modalities such as echocardiography and ECG appear highly desirable. Speckle tracking-derived strain parameters by transthoracic echocardiography have been demonstrated to be more sensitive than conventional echocardiographic parameters for the detection of CA.<sup>32</sup> Characteristic strain pattern of relative apical sparing in CA describes reduced LS at the LV base with progressively increased strain near the LV apex. However, apical sparing was shown insufficient for the detection of CA-AS.<sup>9</sup> This finding is in line with our results and may reflect the haemodynamic load imposed on the left ventricle by severe AS. It could be hypothesized that relative apical sparing becomes manifest only after aortic valve replacement. This, however, needs to be proven in further studies. ECG of CA patients can display a variety of non-specific abnormalities, such as atrial fibrillation, conduction disturbances, and pseudo-infarct pattern. Moreover, affected patients frequently present with low-voltage, which should raise suspicion given the oftentimes heavily thickened myocardium. The VMR is a parameter that combines LV mass and signs of hypertrophy on ECG.<sup>33</sup> Its





**Figure 3** Kaplan–Meier curves of patients with aortic stenosis (AS) with/without cardiac amyloidosis (CA). Concomitant cardiac amyloidosis (CA-AS) was not associated with all-cause mortality (A) or cardiovascular (CV) hospitalization (B) over  $15.3 \pm 7.9$  months following transcatheter aortic valve replacement. One CA-AS patient died prior to transcatheter aortic valve replacement and was therefore excluded from outcome analysis.

diagnostic ability is explained by significantly increased LV mass in CA-AS patients that is, in contrast to isolated AS, not reflected by ECG alterations. We showed here that VMR effectively discriminated between AS and CA-AS (AUC 0.770,  $P = 0.001$ ). Furthermore, we tested SVi for the detection of CA-AS. The underlying idea is related to the fact that CA-AS patients often display low flow pattern, as known from previous reports.<sup>9,34</sup> Indeed, SVi was able to detect CA-AS with an AUC of 0.773. Based on our results, VMR and SVi are useful tools to screen TAVR patients for coexisting CA. In case of CA suspicion, further testing

(<sup>99m</sup>Tc-DPD bone scintigraphy, CMR, blood and urine tests) is indicated.

### Outcome after transcatheter aortic valve replacement

To our knowledge, this is the first trial to investigate the prognostic significance of coexisting CA in a large TAVR cohort. In contrast to previous studies following SAVR,<sup>8,10</sup> CA-AS patients were not found to experience worse outcomes  $15.3 \pm 7.9$  months after

TAVR. It is uncertain whether this also applies to long-term outcome.

## Limitations

The data presented were collected in a single-centre setting. Therefore, a centre-specific bias cannot be excluded. However, the major advantages of limiting data collection to a single centre are (i) adherence to a constant clinical routine, (ii) constant quality of work-up, (iii) and constant follow-up. Unfortunately, out of six patients with Perugini grade 1 uptake on bone scintigraphy, only one patient agreed to perform EMB and was eventually diagnosed with TTR-CA. The remaining five patients declined EMB, which would have been necessary to diagnose CA according to current consensus criteria.<sup>14</sup> Therefore, it cannot be excluded that further CA cases were missed in the present series. Moreover, in one patient with Perugini grade 2 uptake and monoclonal gammopathy of undetermined significance based on bone marrow biopsy, EMB was not performed. Given the known coexistence of TTR-CA and monoclonal protein without AL-CA<sup>14</sup> and the low percentage of AL-CA with Perugini uptake  $\geq 2$ ,<sup>25</sup> this subject was classified as TTR-CA. Unfortunately, mass spectroscopy was not available in our institution for patients undergoing EMB. However, in the two CA subjects with EMB (one TTR, one AL), immunohistochemical analysis did not show ambiguous results, and mass spectroscopy was not essential for an accurate diagnosis according to recent recommendations.<sup>5</sup> The mean age of the present population was  $81 \pm 8.0$  years. Our results may, therefore, not be transferable to younger, low-risk AS populations. Furthermore, AL-CA has an age peak of 60–69 years<sup>35</sup> and may therefore be more prevalent among younger AS patients. Since this cohort mostly comprised cases of TTR-CA, the applicability of VMR and SVi for the screening for AL-CA has yet to be evaluated. The small number of CA cases limited the number of factors that could be included in the multivariate regression analysis and precluded multivariate analysis in the subpopulation with all screening modalities, including CMR. Moreover, the lack of follow-up echo strain data precluded an analysis of whether relative apical sparing becomes overt in CA-AS after valve replacement.

## Conclusions

In the present study, comprehensive screening for CA in AS scheduled for TAVR for the first time revealed not only TTR- but also AL-CA. Most CA patients identified had TTR-CA. Parameters solely based on ECG and echocardiography allow for the identification of the majority of CA-AS. As 15-month survival did not differ significantly between AS with and without CA, TAVR appears beneficial in CA-AS. However, further studies are needed to elucidate potential benefits of TAVR in CA-AS on the long run.

## Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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