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ORIGINAL RESEARCH

Cholesterol Crystal Dissolution Rate of Serum Predicts Outcomes in Patients With Aortic Stenosis Undergoing Transcatheter Aortic Valve Replacement

Baravan Al-Kassou, MD*; Lara Al-Kassou, MD*; Thorsten Mahn, MD; Dieter Lütjohann, PhD; Jasmin Shamekhi, MD; Nicola Willemsen, MD; Sven Thomas Niepmann, MD; Stephan Baldus, MD; Malte Kelm, MD; Georg Nickenig, MD; Eicke Latz, MD; Sebastian Zimmer, MD

BACKGROUND: Aortic stenosis has pathophysiological similarities with atherosclerosis, including the deposition of cholesterol-containing lipoproteins. The resulting cholesterol crystals activate the NLRP3 (NOD-like receptor protein 3) inflammasome, leading to inflammation and cardiovascular diseases. We aimed to investigate the cholesterol crystal dissolution rate (CCDR) of serum in patients with aortic stenosis and to assess the prognostic value of this biomarker.

METHODS AND RESULTS: The study included 348 patients with aortic stenosis undergoing transcatheter aortic valve replacement. The CCDR was measured using flow cytometry to enumerate cholesterol crystals that were added to a serum solution, at baseline and after 2 hours of incubation. Based on the median CCDR, the cohort was stratified into high and low cholesterol crystal dissolvers. The incidence of the primary end point, a composite of 1-year all-cause mortality and major vascular complication, was significantly lower in the high CCDR group (7.3 per 100 person-years) compared with the low CCDR group (17.0 per 100 person-years, P=0.01). This was mainly driven by a lower 1-year mortality rate in patients with a high CCDR (7.3 versus 15.1 per 100 person-years, P=0.04). Unplanned endovascular interventions were significantly less frequent in high cholesterol crystal dissolvers (12.8 versus 22.6 per 100 person-years, P=0.04). Although low-density lipoprotein cholesterol levels were comparable in both groups (101.8±37.3 mg/dL versus 97.9±37.6 mg/dL, P=0.35), only patients with a low CCDR showed a benefit from statin treatment. In multivariate analysis, low CCDR (hazard ratio, 2.21 [95% CI, 0.99–4.92], P=0.04) was significantly associated with 1-year mortality.

CONCLUSIONS: The CCDR is a novel biomarker associated with outcome in patients with aortic stenosis undergoing transcatheter aortic valve replacement. It may provide new insights into patients' anti-inflammatory capacity and additional prognostic information beyond classic risk assessment.

Key Words: calcific aortic stenosis ■ cholesterol crystal dissolution rate ■ cholesterol crystals ■ TAVR

Calcific aortic stenosis (AS) is the most common acquired valvular heart disease in developed countries.[1,2] Its incidence is expected to rise in the coming years due to a growing life expectancy of modern societies.[3] AS is characterized by thickening and calcification of the aortic valve cusps and results

Correspondence to: Sebastian Zimmer, MD, Heart Center Bonn, Department of Medicine II, University Hospital Bonn, Venusberg-Campus 1, 53127 Bonn, Germany. Email: sebastian.zimmer@ukbonn.de

*B. Al-Kassou and L. Al-Kassou contributed equally.

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CLINICAL PERSPECTIVE

What Is New?

- We present a novel assay for quantifying the endogenous cholesterol crystal dissolution rate (CCDR), a major contributor to interleukin-1 driven chronic inflammation, in patients with severe aortic stenosis undergoing transcatheter aortic valve replacement.
- A low CCDR was associated with significantly higher bilateral iliofemoral calcification and increased vascular injury during the transcatheter aortic valve replacement procedure compared with a high CCDR.
- A low CCDR was associated with increased 1-year mortality following transcatheter aortic valve replacement, especially driven by cardiovascular mortality, in comparison with a high CCDR.

What Are the Clinical Implications?

- The CCDR represents a novel biomarker with the potential to provide new insights into the preventative anti-inflammatory capability and additional prognostic information, thus improving the identification of vulnerable patients.

Nonstandard Abbreviations and Acronyms

AS	aortic stenosis
CC	cholesterol crystal
CCDR	cholesterol crystal dissolution rate
PY	person-years
TAVR	transcatheter aortic valve replacement

in an obstruction of left ventricular outflow, leading to heart failure and ultimately to death from cardiovascular causes.[4]

The pathophysiology of AS has several findings in common with atherosclerotic diseases, including chronic inflammation, deposition of lipoproteins, and active calcification.[5–8] Additionally, cumulative effects of genetic risk variants and cardiovascular risk factors as well as lipoprotein and total cholesterol levels play a crucial role in the development of AS.[4,9,10]

Initially, mechanical and shear stress induced dysfunction of valvular endothelial cells leads to the deposition of cholesterol containing lipoproteins and the infiltration of immune cells in the aortic valve cusps.[6,11] If the local cholesterol concentration exceeds its solubility, cholesterol crystals (CC) form and are deposited in the extracellular space. In fact, histopathological examination of resected human aortic valves has revealed that calcific aortic valve cusps abundantly contain CC.[12,13] Moreover, it has been demonstrated that CCs can activate the NLRP3 (NOD-like receptor protein 3) inflammasome in macrophages, resulting in an interleukin-1 driven inflammation that leads to the development of cardiovascular diseases.[14,15] Accordingly, deposits of CC appear to be an early cause rather than a late consequence of inflammation, contributing to the progression of lesion formation and ultimately leading to the development of severe AS.[14]

Currently, the only effective treatment for severe AS is either surgical or transcatheter aortic valve replacement (TAVR). However, valve replacement only removes the left ventricular outflow obstruction and does not modify the underlying pathology. Moreover, several case reports have reported CC embolization after TAVR, affecting various downstream organ systems.[16–18] Thus, despite the distinct improvements in outcomes achieved by these interventions, periprocedural complications and high 1-year mortality rates ranging between 23.7% and 30.7% remain significant concerns, potentially attributed to progressive inflammation and calcification.[19,20] Investigating the role of CC, a main factor of chronic inflammation and the signaling pathway in AS, is therefore of great interest in this context. The aim of the present study was to quantify the endogenous cholesterol crystal dissolution rate (CCDR) in patients with AS undergoing TAVR. We sought to examine whether CCDR is associated with clinical outcomes and suitable for identifying vulnerable patients with increased sclerotic burden at high risk despite valve replacement.

METHODS

Patient Population

From January 2017 to February 2020, 388 patients with severe native AS underwent TAVR with next-generation transcatheter heart valves at the Heart Center Bonn and were included in this study. In this analysis, 40 patients were excluded for whom no valid CCDR measurements could be obtained. Moreover, exclusion criteria included patients with bicuspid or noncalcified aortic valves (n=2), active endocarditis (n=0), valve-in-valve procedures for degenerated aortic valve prostheses (n=11), and patients with an estimated life expectancy <1 year (n=1). All patients underwent a detailed preoperative evaluation including transesophageal echocardiography, computed tomography (CT), and coronary angiography. After evaluation, all cases were discussed within the local interdisciplinary heart team. The study was approved by the local ethics committee of the University of Bonn (No. 077/14). Written and informed consent was obtained from all patients

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before blood collection. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study End Point and Follow-Up

The primary end point of the study was prespecified as a composite of 1-year all-cause mortality and major vascular complications. Key secondary end points included 30-day all-cause mortality, stroke, myocardial infarction, major bleeding complication, and acute kidney injury at 30 days according to the Valve Academic Research Consortium 3 definition criteria as well as cardiovascular and noncardiovascular mortality at 1 year.[21] The prosthesis–patient mismatch was calculated and considered moderate if indexed effective orifice area was ≤0.85 cm^2/m^2 and severe when ≤0.65 cm^2/m^2, as described previously.[22] Furthermore, preinterventional CT scans were analyzed using 3 mensio Structural Heart software (Pie Medical Imaging BV, Maastricht, the Netherlands) to quantify the extent of aortic valve and iliofemoral calcification. The aortic valve calcification was divided into the corresponding sectors of the right, left, and noncoronary cusp. The iliofemoral calcification was measured starting from the femoral artery bifurcation to the aortic bifurcation. As published previously, an adjusted threshold of 550, 300, and 50 Hounsfield units (HU) was used to assess the calcium volume scoring in patients with luminal attenuation of 200 to 500 HU, <200 HU, and >500 HU, respectively.[23] Follow-up data were collected during routine outpatient visits and via standardized telephone interviews with the referring cardiologists or general practitioners.

Blood Sample Collection

After admission to the hospital, but before performing the TAVR procedure, blood samples were obtained from all enrolled patients. The samples were drawn from the central venous catheter and collected in standard tubes (S-Monovette 7.5 mL, Sarstedt AG & Co. KG, Nürnbrecht, Germany). To separate the serum from corpuscular components, the samples were immediately centrifuged at 3000 rpm (1415 *g*) for 10 minutes. Subsequently, the samples were aliquoted and stored at −80 °C in the BioBank Bonn.

Cholesterol Crystal Preparation

The preparation of CCs was carried out from a 2 mg/mL (w/v) cholesterol solution in propan-1-ol (Cholesterol, SIGMA Life Science, St. Louis, MO; Propan-1-ol pure, AppliChem, Darmstadt, Germany, respectively). Crystal formation was induced by adding 1.5× volumes of endotoxin-free water (UltraPure DNase/RNase-Free Distilled Water, LIFE Technologies Limited, Carlsbad, CA). The prepared CCs were left to rest for 24 hours at constant room temperature and then carefully dried at 30 °C in a concentrator (Concentrator 5301, Eppendorf, Hamburg, Germany). The CCs were resuspended in PBS (pH 7.4, Life Technologies Europe BV, Bleiswijk, Netherlands) to obtain a concentration of 10 mg/mL (w/v). Bovine albumin (bovine serum albumin fraction V, GE Healthcare, Pasching, Austria) was added to achieve a concentration of 1% after sonification (RK 100, Bandelin Electronic, Berlin, Germany) for 3 minutes. CCs were stored in sterile glass tubes at 4 °C. Before analysis, the suspension was vortexed for at least 1 minute. Counting beads and size calibration beads were used for validation of crystal concentration and size in all samples (Count Bright Absolute Counting Beads and Flow Cytometry Size Calibration Kit, Life Technologies Corporation, Eugene, OR). Finally, we obtained a concentration of $3.5x10^6$ CCs per μL at 3 to 7 μm. Crystal quantification was validated by an independent electric sensing zone method, using a Coulter Counter with a threshold of 3 μm (Coulter Counter Z2, Beckman Coulter Life Science, Indianapolis, IN). Pearson correlation showed a high agreement between the flow cytometry and electric sensing zone methods (Pearson coefficient, 0.949).

Cholesterol Crystal Dissolution Rate

Flow cytometry was performed to enumerate the CCs (FACSCalibur, Becton and Dickinson Company, Franklin Lakes, NJ). The obtained data were subsequently analyzed with FlowJo software (FlowJo V10, FlowJo LLC, Ashland, OR) using forward and side scatter characteristics to identify CCs. A gating strategy that focused on the most intense region, representing 50% of all crystals in a range between 3 and 5 μm, was used to exclude counts caused by serum. This approach resulted in an exclusion of large crystal agglomerates and smaller fragments, as shown in Figure S1. For the analysis of the CCDR, that is time and serum concentration dependent, 140 μg of the CC suspension and 10 μL of counting beads were added to 150 μL of sample serum to achieve a final 50% serum solution. The mixture was incubated for 2 hours at 37 °C with constant shaking. All measurements were done as quintuplets. The assessment of CCDR was performed at baseline and after 2 hours of incubation, indicating the reduction of CC as percentage change. To internally validate our assay, several tests were conducted. Regarding the assessment of consistency and reproducibility of the quantitative measurements, an intraclass correlation coefficient showed a good agreement with a Cronbach alpha of 0.713.

Statistical Analysis

Continuous variables are presented as means with SD if normally distributed and as median with interquartile

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range if not normally distributed. The Kolmogorov–Smirnov test was used to assess normal distribution of continuous variables. Continuous variables were tested with the Student's *t* tests or Mann–Whitney *U* test, depending on the distribution. Categorical variables are given as absolute numbers and percentages. Differences in categorical variables were assessed using Fisher's exact test. Correlation analysis was performed using Pearson's correlation coefficient. Linear regression analysis was conducted to visualize the correlation between CT assessed calcium volume and cholesterol crystal dissolution rate of serum. Primary and secondary outcome according to the CCDR classification was estimated by the Kaplan–Meier method, and log-rank test was used to determine statistical significance. Statistically significant predictors of 1-year all-cause mortality were identified by first including the parameters in a univariate analysis and subsequently entering the significant predictors with a *P* value ≤0.05 in a Cox proportional hazards model. Statistical analyses were performed with SPSS version 28 (IBM Corporation, Somer, NY) and Stata version 14.2 (StataCorp LLC, College Station, TX). Statistical significance was considered as a 2-tailed probability value ≤0.05. All authors vouch for the data and analyses.

RESULTS

The study cohort comprised 348 patients with AS undergoing TAVR, from whom valid CCDR measurements were obtained. The baseline characteristics of all patients are summarized in Table 1. The study population was 47.7% female and had a mean age of 80.9±6.2 years. The median of the Society of Thoracic Surgeons predicted risk of mortality (2.9%, interquartile range, 2.1–4.6%) and the median of EuroSCORE II (3.3%, interquartile range, 2.0–5.8%) indicated a low surgical risk for the overall cohort. The incidence of coronary and peripheral artery disease was 57.8% and 51.7%, respectively. Predominant cardiovascular risk factors included arterial hypertension (83.9%), hypercholesterolemia (62.6%), and diabetes (30.7%).

According to the flow cytometry-based analysis, the dissolution of CCs varied from no dissolution to a dissolution of >70%. The study cohort was stratified into high and low CC dissolvers based on the median CCDR. A low CC dissolution was associated with a higher rate of atrial fibrillation (54.0% versus 41.9%, *P*=0.03). Accordingly, the indication for permanent oral anticoagulation was significantly higher in patients with a low CCDR (58.3% versus 42.2%, *P*=0.004), whereas a dual antiplatelet therapy was more common in patients with a high CCDR (54.1% versus 38.6%, *P*=0.005), as shown in Table 2. With respect to the implanted next-generation transcatheter heart valves, the proportion of balloon-expandable and self-expanding heart valves was comparable between high and low CC dissolvers (48.8% versus 56.8%, *P*=0.16; 51.2% versus 43.2%, *P*=0.16, respectively).

Although the serum concentration of low-density lipoprotein (LDL) cholesterol (101.8±37.3 mg/dL versus 97.9±37.6 mg/dL, *P*=0.35) and total cholesterol (158.1±43.8 mg/dL versus 154.1±40.2 mg/dL, *P*=0.41), as well as previously established use of statins (76.2% versus 83.0%, *P*=0.14), were comparable in patients with high and low CCDR, the lipid-lowering therapy showed a different efficacy in both groups: statin treatment was associated with significantly reduced levels of LDL (94.1±35.7 mg/dL versus 116.9±41.5 mg/dL, *P*=0.003) and total cholesterol (149.8±37.1 mg/dL versus 174.9±48.4 mg/dL, *P*=0.003), mainly in patients with low CCDR, as presented in Figure 1.

Primary End Point

The composite of 1-year all-cause mortality and major vascular complications occurred in 12 patients (7.3 per 100 person-years [PY]) with a high CCDR, compared with 27 patients with a low CCDR (17.0 per 100 PY, *P*=0.01), as presented in Figure 2 and Table 3. The significantly lower rate of the primary end point in high CC dissolvers was mainly driven by lower rates of 1-year mortality as compared with low CC dissolvers (7.3 versus 15.1 per 100 PY, *P*=0.04) (Figure 3). Interestingly, noncardiovascular mortality was comparable between both groups (5.5 versus 5.0 per 100 PY, *P*=0.81), whereas patients with a high CCDR showed significantly lower rates of cardiovascular mortality at 1 year (1.8 versus 10.1 per 100 PY, *P*=0.003), as indicated in Figure 4. The rate of major vascular complication was numerically higher in patients with a low CCDR but did not reach significance (3.8 versus 0.6 per 100 PY, *P*=0.06).

Key Secondary End Points

In the overall cohort, both stroke and myocardial infarction occurred in only 2 patients, with no significant difference between patients with high and low CCDR (*P*=0.99). Moreover, the incidence of major bleeding complications was comparable in both groups (2.4 versus 2.8 per 100 PY, *P*=0.87). Acute kidney injury was observed in 3 high CC dissolvers and 1 low CC dissolver (1.8 versus 0.6 per 100 PY, *P*=0.30). CT-based calcium volume assessment revealed comparable total aortic valve calcification between both groups but significantly higher noncoronary cusp calcification in low CC dissolvers (329 mm^3 versus 264 mm^3, *P*=0.04) when divided into the corresponding leaflet sectors (Table 4). Moreover, significantly higher right as well as left iliofemoral calcification was detectable in patients with a low CCDR (1280 mm^3 versus 1083 mm^3, *P*=0.03;

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Table 1. Baseline Characteristics of the Patients

	Overall cohort (n=348)	High CCDR (n=172)	Low CCDR (n=176)	*P* value
Age, y	80.9±6.2	81.0±6.0	80.8±6.5	0.67
Body mass index, kg/m^2	26.5±4.7	26.5±4.6	26.6±4.8	0.82
Female sex, %	166 (47.7)	84 (48.8)	82 (46.6)	0.75
EuroSCORE II, %	3.3 (2.0–5.8)	3.3 (2.0–6.0)	3.2 (1.8–5.6)	0.54
Society of Thoracic Surgeons predicted risk of mortality, %	2.9 (2.1–4.6)	3.0 (2.1–5.1)	2.9 (2.1–4.4)	0.34
Left ventricular ejection fraction, %	53.9±11.6	55.0±10.8	52.7±12.4	0.08
Chronic obstructive pulmonary disease, %	50 (14.4)	25 (14.5)	25 (14.2)	1.0
Coronary artery disease, %	201 (57.8)	96 (55.8)	105 (59.7)	0.52
Myocardial infarction, %	37 (10.6)	22 (12.8)	15 (8.5)	0.23
Previous percutaneous coronary intervention, %	134 (38.5)	66 (38.4)	68 (38.6)	1.0
Previous cardiac surgery, %	37 (10.6)	21 (12.2)	16 (9.1)	0.39
Atrial fibrillation, %	167 (48.0)	72 (41.9)	95 (54.0)	0.03
Stroke, %	35 (10.1)	17 (9.9)	18 (10.2)	1.0
Arterial hypertension, %	292 (83.9)	138 (80.2)	154 (87.5)	0.08
Diabetes, %	107 (30.7)	51 (29.7)	56 (31.8)	0.73
Hypercholesterolemia, %	218 (62.6)	106 (61.6)	112 (63.6)	0.74
Peripheral artery disease, %	180 (51.7)	90 (52.3)	90 (51.1)	0.83
Creatinine, mg/dL	1.1 (0.9–1.4)	1.1 (0.9–1.4)	1.1 (0.9–1.4)	0.78
Hemoglobin, g/dL	11.9±1.7	12.1±1.8	11.8±1.6	0.19
Leukocytes, g/L	7.1 (6.0–8.6)	7.0 (6.0–8.5)	7.3 (5.9–8.6)	0.60
Troponin, pg/mL	24.0 (16.7–39.1)	23.6 (6.5–38.5)	25.0 (17.0–39.7)	0.40
Low-density lipoprotein cholesterol, mg/dL	99.8±37.4	101.8±37.3	97.9±37.6	0.35
High-density lipoprotein cholesterol, mg/dL	55.0 (43.0–67.0)	56.0 (44.0–71.0)	54.0 (41.3–64.8)	0.07
Total cholesterol, mg/dL	156.0±42.0	158.1±43.8	154.1±40.2	0.41
Triglyceride, mg/dL	96.0 (69.3–134.8)	90.5 (66.0–133.3)	99.0 (73.8–139.5)	0.18
C-reactive protein, mg/L	2.8 (1.0–6.8)	2.9 (0.9–6.6)	2.7 (1.02–7.0)	0.48
Procalcitonin, µg/L	0.05 (0.03–0.07)	0.05 (0.03–0.07)	0.04 (0.03–0.07)	0.24
Chronic renal failure, %	161 (46.3)	80 (46.5)	81 (46.0)	0.91
Dialysis, %	4 (1.1)	2 (1.2)	2 (1.1)	1.0

CCDR indicates cholesterol crystal dissolution rate.

1292 mm^3 versus 1043 mm^3, *P*=0.04, respectively), as shown in Figure S2. Remarkably, the rate of unplanned endovascular intervention for access-related vascular injury was significantly higher in patients with a low CCDR, as compared with patients with a high CCDR (22.6 versus 12.8 per 100 PY, *P*=0.04). The overall 30-day all-cause mortality rate was 4.3 per 100 PY and did not differ significantly between the groups (*P*=0.12).

Predictors of Clinical Outcomes

Univariate and multivariate analyses revealed that only albumin (hazard ratio [HR], 0.88 [95% CI, 0.81–0.96], *P*=0.005) and a low CCDR (HR, 2.21 [95% CI, 0.99–4.92], *P*=0.04) were statistically significantly associated with 1-year all-cause mortality, as shown in Table 5. There was no association of patient age (HR, 1.04 [95% CI, 0.99–1.11], *P*=0.14), sex (HR, 0.91 [95% CI, 0.47–1.78], *P*=0.80), left ventricular ejection fraction (HR, 0.99 [95% CI, 0.96–1.02] *P*=0.51), or C-reactive protein (HR, 1.0 [95% CI, 1.0–1.02], *P*=0.19) with outcome in the univariate analyses. Moreover, although significant in the univariate analysis, atrial fibrillation (HR, 0.69 [95% CI, 0.20–2.37], *P*=0.56) as well as oral anticoagulant therapy (HR, 2.48 [95% CI, 0.70–8.86], *P*=0.16) showed no significant association with the outcome in multivariate analyses.

DISCUSSION

Contrary to the long-held assumption of a mainly mechanically driven process, the pathophysiology of AS is now established as a stress-induced and cellular-regulated signaling pathway.[11] Cardiovascular risk factors contribute to chronic inflammation, leading to fibro- and osteoblastic activation of valvular interstitial cells with subsequent calcification.[4,6,24,25] However,

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Table 2. Prior Medical Treatment and Procedural Data, %

	Overall cohort (n=348)	High CCDR (n=172)	Low CCDR (n=176)	*P* value
Prior medical treatment				
Dual antiplatelet therapy, %	161 (46.3)	93 (54.1)	68 (38.6)	0.005
Oral anticoagulants, %	175 (50.4)	73 (42.2)	102 (58.3)	0.004
Beta blocker, %	216 (65.1)	107 (64.1)	109 (66.1)	0.73
Angiotensin-converting enzyme inhibitor, %	125 (37.7)	65 (38.9)	60 (36.4)	0.65
Angiotensin receptor blocker, %	98 (29.5)	52 (31.1)	46 (27.9)	0.55
Calcium antagonist, %	94 (28.3)	41 (24.6)	53 (32.1)	0.14
Diuretic, %	227 (68.4)	115 (68.9)	112 (67.6)	0.91
Statin, %	277 (79.6)	131 (76.2)	146 (83.0)	0.14
Ezetimibe, %	21 (6.3)	12 (7.2)	9 (5.5)	0.65
Implanted transcatheter heart valves				
Balloon-expandable heart valves, %	184 (52.9)	84 (48.8)	100 (56.8)	0.16
Self-expanding heart valves, %	164 (47.1)	88 (51.2)	76 (43.2)	0.16
Procedural results				
Moderate or severe paravalvular leak, %	3 (0.9)	1 (0.6)	2 (1.1)	1.0
Moderate PPM (iEOA ≤0.85 cm^2/m^2)	9 (2.6)	4 (2.3)	5 (2.8)	1.0
Severe PPM (iEOA ≤0.65 cm^2/m^2)	0 (0)	0 (0)	0 (0)	1.0

CCDR indicates cholesterol crystal dissolution rate; iEOA, indexed effective orifice area; and PPM, prosthesis–patient mismatch.

modification and medical treatment show limited efficacy in preventing AS progression.[26–28] This may be because these risk factors merely reflect individual health conditions, rather than providing insights into patient's capability to react to stressors.

In this study, we present a novel assay for measuring the serum capacity to dissolve CCs. As previously shown, CCs are not only the main components of atherosclerotic plaques in vessels but also a main constituent of calcific aortic valve cusps and a major endogenous danger signal inducing inflammation.[12–14] Accordingly, the capacity to dissolve CCs reflects the specific capability of individuals to prevent chronic inflammation, even beyond aortic valve replacement. In fact, our results show that patients with a low CCDR had a 2-fold increased risk of 1-year all-cause mortality compared with patients with a high CCDR (*P*=0.04). This association was mainly driven by cardiovascular death, which was 5-fold higher in low CC dissolvers (*P*=0.003), whereas noncardiovascular mortality rates were similar between both groups (*P*=0.78). The overall 30-day mortality was 4.0%, compared with predicted 30-day mortalities of 3.3% and 2.9% according to the EuroSCORE II and Society of Thoracic Surgeons predicted risk of mortality, respectively. This finding aligns with several studies that have shown that both scores underestimate the risk in patients undergoing TAVR.[29,30] Notably, the predicted mortality was slightly overestimated in patients with a high CCDR, whereas it was significantly underestimated in patients with a

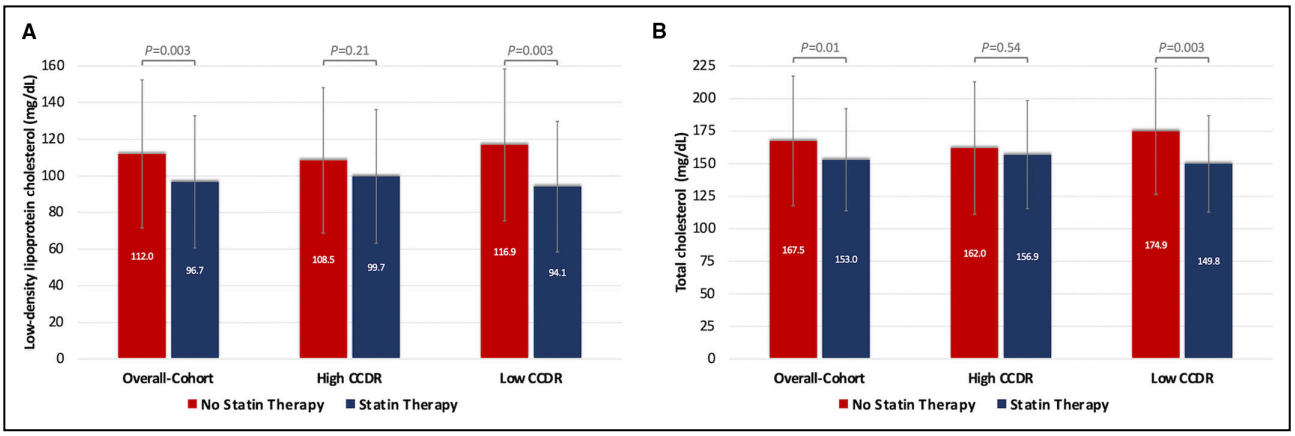

Figure 1. LDL and total cholesterol levels according to CCDR.
A statin treatment was associated with significantly reduced LDL cholesterol (**A**) and total cholesterol (**B**) levels only in patients with a low CCDR. CCDR indicates cholesterol crystal dissolution rate; and LDL, low-density lipoprotein.

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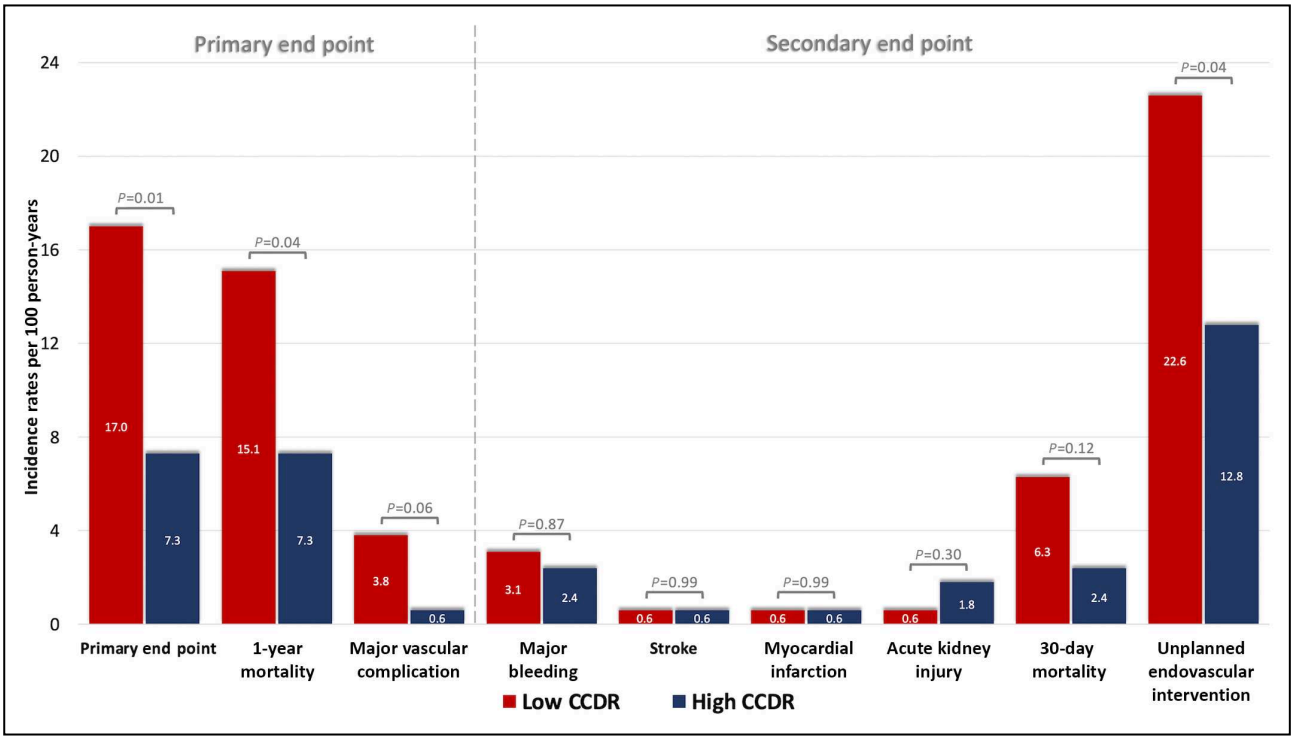

Figure 2. Clinical outcomes according to CCDR.
The primary end point, a composite of 1-year all-cause mortality and major vascular complication, was significantly less frequent in patients with a high CCDR, mainly driven by lower rates of mortality. The need for unplanned endovascular interventions was more frequent in patients with a low CCDR, whereas there was no significant difference in the occurrence of stroke or myocardial infarction. CCDR indicates cholesterol crystal dissolution rate.

low CCDR. This discrepancy, especially in patients with a low CCDR, supports our hypothesis that CCDR may provide additional prognostic information beyond classic risk assessment. Regarding procedure-specific complication, major vascular complications were numerically 5 times more frequent in patients with low

Table 3. Clinical End Points

	Overall cohort (n=348)		High CCDR (n=172)		Low CCDR (n=176)		
	n (%)	IR/100 PY	n (%)	IR/100 PY	n (%)	IR/100 PY	*P* value
Primary end point	39 (11.2)	12.0	12 (7.0)	7.3	27 (15.3)	17.0	0.01*
1-year all-cause mortality	36 (10.3)	11.1	12 (7.0)	7.3	24 (13.6)	15.1	0.04*
Cardiovascular mortality	19 (5.5)	5.9	3 (1.7)	1.8	16 (9.1)	10.1	0.003*
Noncardiovascular mortality	17 (4.9)	5.3	9 (5.2)	5.5	8 (4.5)	5.0	0.78
Major vascular complication	7 (2.0)	2.2	1 (0.6)	0.6	6 (3.4)	3.8	0.06
Key secondary end points							
Major bleeding	9 (2.6)	2.8	4 (2.3)	2.4	5 (2.8)	3.1	0.87
Minor vascular complication	53 (15.2)	16.4	26 (15.1)	15.8	27 (15.3)	17.0	0.95
Stroke	2 (0.6)	0.6	1 (0.6)	0.6	1 (0.6)	0.6	0.99
Myocardial infarction	2 (0.6)	0.6	1 (0.6)	0.6	1 (0.6)	0.6	0.99
Acute kidney injury	4 (1.1)	1.2	3 (1.7)	1.8	1 (0.6)	0.6	0.30
Unplanned endovascular interventions	57 (16.4)	17.6	21 (12.2)	12.8	36 (20.5)	22.6	0.04*
New permanent pacemaker	25 (7.2)	7.7	14 (8.1)	8.5	11 (6.3)	6.9	0.51
30-d all-cause mortality	14 (4.0)	4.3	4 (2.3)	2.4	10 (5.7)	6.3	0.12

CCDR indicates cholesterol crystal dissolution rate; IR, incidence rate; and PY, person-years.
*A 2-tailed probability value $P \leq 0.05$ was considered statistically significant.

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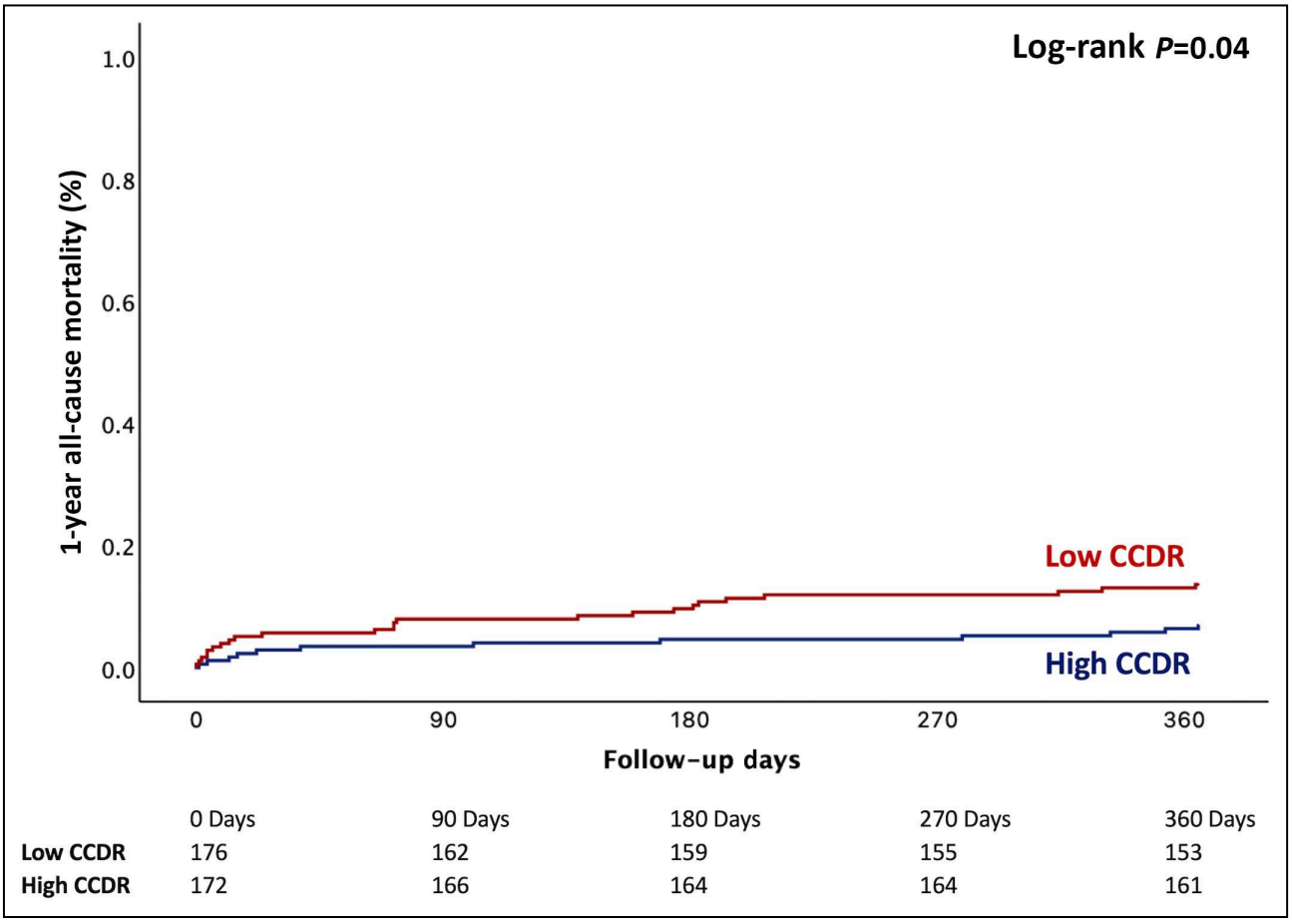

Figure 3. Kaplan–Meier survival analysis for one-year mortality according to CCDR.
Kaplan–Meier survival analyses showed that a low CCDR was associated with significantly higher 1-year mortality rates. The red line represents patients with a low CCDR, the blue line is for patients with high CCDR. CCDR indicates cholesterol crystal dissolution rate.

CCDR, although not reaching significance (P=0.06). Contrast-enhanced CT scans revealed a significant ≈20% increase in bilateral iliofemoral calcification in the low CCDR group (P≤0.04). Accordingly, the need for unplanned endovascular interventions for access-related vascular injury was 68% higher in patients with a low as compared with a high CCDR (P=0.04). This may indicate an increased atherosclerotic burden in these patients, even if this is not reflected in classic clinical parameters such as coronary or peripheral artery disease. In regard to other procedure-related factors that may affect late outcomes in patients undergoing TAVR, such as paravalvular leaks, prosthesis-patient mismatch, and permanent pacemaker implantation, no differences were observed between high and low CC dissolvers (P≥0.54).[22,31,32]

In general, lipid-lowering strategies using β-hydroxy β-methylglutaryl-CoA reductase and proprotein convertase subtilisin/kexin type 9 inhibitors to reduce LDL cholesterol levels are among the most effective interventions to modify the cardiovascular risk profile.[33,34] According to the current guidelines of the European Society of Cardiology for the management of dyslipidemias, the greatest possible reduction of LDL levels is recommended to prevent cardiovascular diseases, especially in high-risk and very high-risk patients.[35] However, in patients with mild-to-moderate AS, several randomized trials have failed to show a beneficial effect of a lipid-lowering therapy in slowing the progression of the stenosis.[28,36,37] This might be explained by the fact that new biomarkers of the cholesterol metabolism, such as the cholesterol efflux capacity, are more sensitive in predicting cardiovascular events and may be more promising targets for AS treatment.[38,39] In our study, LDL-cholesterol levels were similar in both low and high CCDR patients (P=0.35). Interestingly, a statin treatment was associated with significantly reduced LDL- and total cholesterol levels only in the low CCDR group (P=0.003).

Although we were able to demonstrate a clear association between CCDR and clinical outcomes in patients with AS undergoing TAVR, a causality cannot be unequivocally assumed based on these results, as the pathophysiological mechanisms underlying

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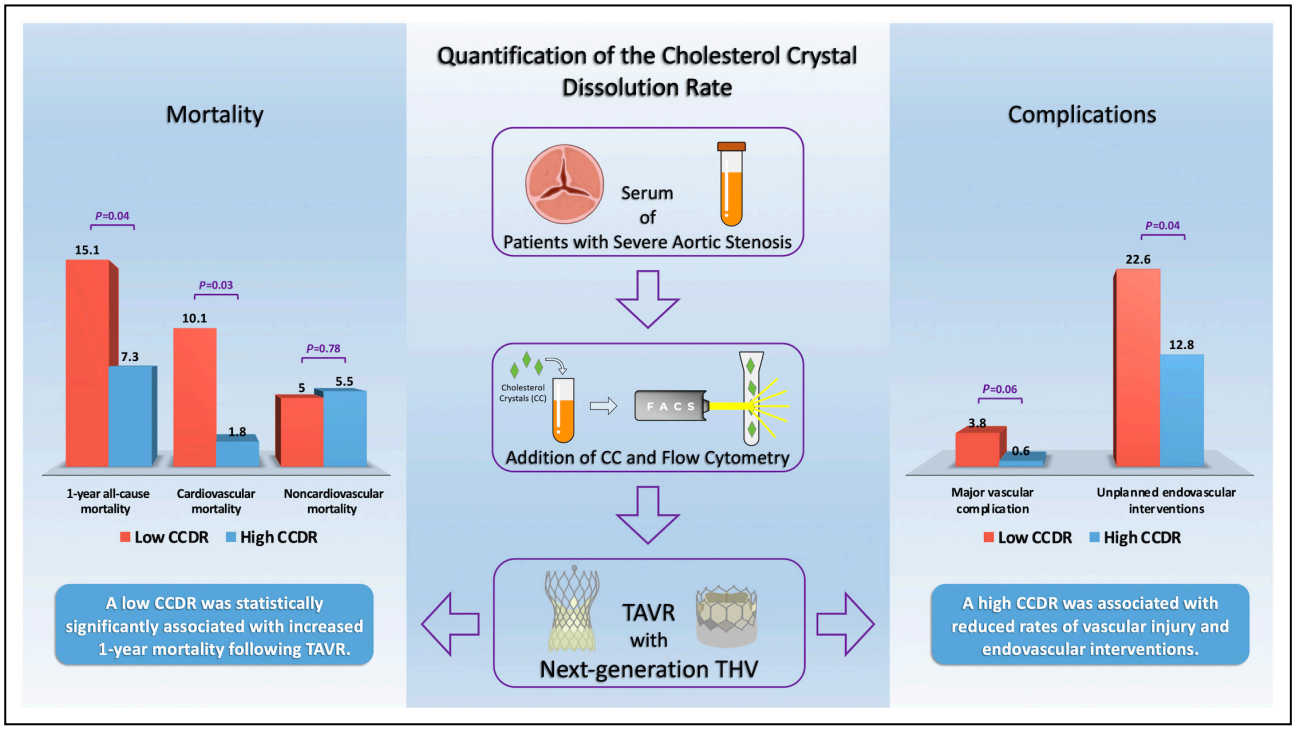

Figure 4. Cholesterol crystal dissolution rate of serum in patients with severe aortic stenosis.
Quantification of the serum rate to dissolve cholesterol crystals predicts outcome in patients with aortic stenosis undergoing TAVR. CC indicates cholesterol crystals; CCDR, cholesterol crystal dissolution rate; TAVR, transcatheter aortic valve replacement; and THV, transcatheter heart valves.

CCDR have not yet been elucidated. In previous studies using size-exclusion filters, our research group located the main constituents of the CCDR at around 300 kilodaltons. We assume that these factors bind and degrade CC, as repetitive addition and incubation of CC depletes the serum and reduces its dissolving capacity.[40,41] Further investigation in this area to elucidate the mechanisms and contributing factors of CCDR may lead to the identification of novel anti-inflammatory pathways that can improve patient outcome both in identifying vulnerable patients and enabling targeted therapeutic intervention. Remarkably, we have previously demonstrated that pharmaceutical interventions with ursodeoxycholic acid and 2-hydroxypropyl-β-cyclodextrin to increase the solubility of cholesterol and thereby decreasing accumulation and deposition of CC can prevent and reverse murine atherosclerosis.[40,41] Moreover, in preliminary experiments, we have shown that pharmacologically increasing the solubility of cholesterol with 2-hydroxypropyl-β-cyclodextrin reduces the development of AS in mice.[42] 2-hydroxypropyl-β-cyclodextrin is a substance approved by the Food and Drug Administration and the European Medicines Agency to solubilize and entrap lipophilic pharmaceutical agents for therapeutic delivery in humans.[43,44] Given its widespread use and established safety profile in humans, 2-hydroxypropyl-β-cyclodextrin can easily be tested as a potential therapeutic option in clinical trials. In such a case, the CCDR could be used to monitor the therapeutic efficacy of the tested substances in increasing cholesterol solubility and consequently reducing

Table 4. Computed Tomography Assessed Calcium Volume (mm^3)

	Overall cohort (n=348)	High CCDR (n=172)	Low CCDR (n=176)	*P* value
Right iliofemoral calcification	1213 (571–2204)	1083 (562–1901)	1280 (656–2809)	0.03
Left iliofemoral calcification	1167 (526–2012)	1043 (489–1785)	1292 (569–2183)	0.04
Total aortic valve calcification	673 (409–990)	636 (403–982)	732 (421–1013)	0.24
Noncoronary cusp calcification	287 (160–446)	264 (140–400)	329 (171–472)	0.04
Left coronary cusp calcification	164 (78–314)	184 (79–308)	161 (77–316)	0.44
Right coronary cusp calcification	178 (73–347)	173 (68–323)	192 (85–367)	0.99

CCDR indicates cholesterol crystal dissolution rate.

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Table 5. Predictors of 1-Year Mortality

	Univariable analysis			Multivariable analysis		
	P value	HR	95% CI	*P* value	HR	95% CI
Age	0.14	1.04	0.99–1.11			
Female sex	0.80	0.91	0.47–1.78			
EuroSCORE II	0.03	1.09	1.0–1.19	0.73	1.02	0.90–1.15
Society of Thoracic Surgeons predicted risk of mortality	0.05	1.67	1.0–2.76	0.15	1.59	0.85–2.98
Left ventricular ejection fraction	0.51	0.99	0.96–1.02			
Troponin	0.34	1.0	1.0–1.01			
C-reactive protein	0.19	1.0	1.0–1.02			
Low-density lipoprotein cholesterol	0.26	0.99	0.98–1.0			
High-density lipoprotein cholesterol	0.03	0.98	0.95–1.0	0.29	0.99	0.96–1.01
Dyslipidemia	0.08	0.56	0.29–1.08	0.44	0.74	0.35–1.57
Atrial fibrillation	0.04	2.06	1.04–4.01	0.56	0.69	0.20–2.37
Oral anticoagulants	0.05	2.00	0.99–4.05	0.16	2.48	0.70–8.86
Dual antiplatelet therapy	0.04	0.48	0.23–0.97	0.91	1.12	0.18–7.06
Albumin	0.001	0.88	0.81–0.95	0.005	0.88	0.81–0.96
Low CCDR	0.04	2.04	1.02–4.07	0.04	2.21	0.99–4.92

CCDR indicates cholesterol crystal dissolution rate.

lesion development in calcific valves and vessels. The identification of "druggable" pathobiological pathways driving inflammation and stenosis development would have a significant impact, especially in patients with AS, as surgical valve replacement and TAVR are currently the only available therapeutic options.[45]

In the present study, we have quantified the CCDR once per patient before the TAVR procedure. Whether the CCDR changes over time or is influenced by pharmaceutical interventions such as β-hydroxy β-methylglutaryl-CoA reductase and proprotein convertase subtilisin/kexin type 9 inhibitors remains unclear. Furthermore, the effects of CCDR on CCs in lesions of calcific aortic valve cusps are beyond the scope of our work, as we investigated patients with preexisting severe AS undergoing TAVR. However, several case series have reported CC embolization caused by CC showers after TAVR that affects different organ systems, including the peripheral vasculature, kidneys, and brain.[16–18] We assume that a high CCDR may protect patients from CC embolization and the associated long-term damage to organ systems. Moreover, a high CCDR may increase the preventative capability of individuals to react to inflammatory stress, leading to a better long-term outcome. In this context, CCDR may also have potential as a clinical marker for worse cardiovascular outcomes in other patient populations, such as patients with coronary heart disease.

Limitations

Limitations of the current assay include the large sample volume of 150 µL needed per replicate. Moreover, it is critical to prepare and use homogenous CCs and to strictly observe the incubation times to avoid falsified CCDR results. Despite the comprehensive clinical data collection of patients, some factors such as left ventricular outflow tract calcification, which may affect later outcomes, were not incorporated into our study.[46] The clinical results are limited by the observational character of our single-center experience. Further validation of the CCDR assay in larger and independent collectives and elucidation of the mechanisms and contributing factors of CCDR is warranted.

CONCLUSIONS

This is the first study to examine the cholesterol crystal dissolution rate of serum in patients with AS undergoing TAVR. We have demonstrated that low CCDR is associated with increased calcification and 1-year mortality following TAVR using a simple and quick test that is highly reproducible and requires only minimal equipment.

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Affiliations

Heart Center, Department of Medicine II (B.A., L.A., T.M., J.S., N.W., S.T.N., G.N., S.Z.), and Institute of Clinical Chemistry und Clinical Pharmacology (D.L.), University Hospital Bonn, Bonn, Germany; Department of Cardiology, Heart Center, University of Cologne, Germany (S.B.); Division of Cardiology, University Hospital of Duesseldorf, Germany (M.K.); CARID, Cardiovascular Research Institute Duesseldorf, Germany (M.K.); Institute of Innate Immunity, University Hospitals Bonn, Bonn, Germany (E.L.); German Center of Neurodegenerative Diseases (DZNE), Bonn, Germany (E.L.); and Department of Infectious Diseases and Immunology, UMass Medical School, Worcester, MA (E.L.).

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Supplemental Material

Figures S1–S2

REFERENCES

1. Nkomo VT, Gardin JM, Skelton TN, Gottdiener JS, Scott CG, Enriquez-Sarano M. Burden of valvular heart diseases: a population-based study. *Lancet*. 2006;368:1005–1011. doi: 10.1016/S0140-6736(06)69208-8
2. Iung B, Vahanian A. Epidemiology of valvular heart disease in the adult. *Nat Rev Cardiol*. 2011;8:162–172. doi: 10.1038/nrcardio.2010.202
3. Osnabrugge RL, Mylotte D, Head SJ, Van Mieghem NM, Nkomo VT, LeReun CM, Bogers AJ, Piazza N, Kappetein AP. Aortic stenosis in the elderly: disease prevalence and number of candidates for transcatheter aortic valve replacement: a meta-analysis and modeling study. *J Am Coll Cardiol*. 2013;62:1002–1012. doi: 10.1016/j.jacc.2013.05.015
4. Stewart BF, Siscovick D, Lind BK, Gardin JM, Gottdiener JS, Smith VE, Kitzman DW, Otto CM. Clinical factors associated with calcific aortic valve disease. Cardiovascular health study. *J Am Coll Cardiol*. 1997;29:630–634. doi: 10.1016/s0735-1097(96)00563-3
5. Dweck MR, Boon NA, Newby DE. Calcific aortic stenosis: a disease of the valve and the myocardium. *J Am Coll Cardiol*. 2012;60:1854–1863. doi: 10.1016/j.jacc.2012.02.093
6. Otto CM, Kuusisto J, Reichenbach DD, Gown AM, O'Brien KD. Characterization of the early lesion of 'degenerative' valvular aortic stenosis. Histological and Immunohistochemical Studies. *Circulation*. 1994;90:844–853. doi: 10.1161/01.cir.90.2.844
7. Lindman BR, Clavel MA, Mathieu P, Iung B, Lancellotti P, Otto CM, Pibarot P. Calcific aortic stenosis. *Nat Rev Dis Primers*. 2016;2:16006. doi: 10.1038/nrdp.2016.6
8. O'Brien KD, Shavelle DM, Caulfield MT, McDonald TO, Olin-Lewis K, Otto CM, Probstfield JL. Association of angiotensin-converting enzyme with low-density lipoprotein in aortic valvular lesions and in human plasma. *Circulation*. 2002;106:2224–2230. doi: 10.1161/01.cir.0000035655.45453.d2
9. Siu SC, Silversides CK. Bicuspid aortic valve disease. *J Am Coll Cardiol*. 2010;55:2789–2800. doi: 10.1016/j.jacc.2009.12.068
10. Aronow WS, Ahn C, Kronzon I, Goldman ME. Association of coronary risk factors and use of statins with progression of mild valvular aortic stenosis in older persons. *Am J Cardiol*. 2001;88:693–695. doi: 10.1016/s0002-9149(01)01821-5
11. Goody PR, Hosen MR, Christmann D, Niepmann ST, Zietzer A, Adam M, Bonner F, Zimmer S, Nickenig G, Jansen F. Aortic valve stenosis: from basic mechanisms to novel therapeutic targets. *Arterioscler Thromb Vasc Biol*. 2020;40:885–900. doi: 10.1161/ATVBAHA.119.313067
12. Rajamannan NM, Bonow RO, Rahimtoola SH. Calcific aortic stenosis: an update. *Nat Clin Pract Cardiovasc Med*. 2007;4:254–262. doi: 10.1038/ncpcardio0827
13. Kuusisto J, Rasanen K, Sarkioja T, Alarakkola E, Kosma VM. Atherosclerosis-like lesions of the aortic valve are common in adults of all ages: a necropsy study. *Heart*. 2005;91:576–582. doi: 10.1136/hrt.2004.036848
14. Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, Abela GS, Franchi L, Nunez G, Schnurr M, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature*. 2010;464:1357–1361. doi: 10.1038/nature08938
15. Janoudi A, Shamoun FE, Kalavakunta JK, Abela GS. Cholesterol crystal induced arterial inflammation and destabilization of atherosclerotic plaque. *Eur Heart J*. 2016;37:1959–1967. doi: 10.1093/eurheartj/ehv653
16. Kadoya Y, Zen K, Takigami M, Yamano M, Yamano T, Nakamura T, Matoba S. Multiple small bowel perforations associated with cholesterol crystal embolization after transcatheter aortic valve replacement. *JACC Cardiovasc Interv*. 2020;13:1831–1832. doi: 10.1016/j.jcin.2020.04.030
17. Devy L, Brunet-Possenti F; Images In Clinical Medicine. Cholesterol embolization after transcatheter aortic-valve replacement. *N Engl J Med*. 2016;375:e25. doi: 10.1056/NEJMicm1514550
18. Lazareth H, Karras A. Cholesterol crystal embolization after transcatheter aortic-valve replacement. *N Engl J Med*. 2019;381:655. doi: 10.1056/NEJMicm1815615
19. Makkar RR, Fontana GP, Jilaihawi H, Kapadia S, Pichard AD, Douglas PS, Thourani VH, Babaliaros VC, Webb JG, Herrmann HC, et al. Transcatheter aortic-valve replacement for inoperable severe aortic stenosis. *N Engl J Med*. 2012;366:1696–1704. doi: 10.1056/NEJMoa1202277
20. Holmes DR Jr, Brennan JM, Rumsfeld JS, Dai D, O'Brien SM, Vemulapalli S, Edwards FH, Carroll J, Shahian D, Grover F, et al. Clinical outcomes at 1 year following transcatheter aortic valve replacement. *JAMA*. 2015;313:1019–1028. doi: 10.1001/jama.2015.1474
21. Varc-3 Writing C, Genereux P, Piazza N, Alu MC, Nazif T, Hahn RT, Pibarot P, Bax JJ, Leipsic JA, Blanke P, et al. Valve academic research consortium 3: updated endpoint definitions for aortic valve clinical research. *J Am Coll Cardiol*. 2021;77:2717–2746. doi: 10.1016/j.jacc.2021.02.038
22. Sa MP, Jacquemyn X, Van den Eynde J, Tasoudis P, Dokollari A, Torregrossa G, Sicouri S, Clavel MA, Pibarot P, Ramlawi B. Impact of prosthesis-patient mismatch after transcatheter aortic valve replacement: meta-analysis of Kaplan-Meier-derived individual patient data. *JACC Cardiovasc Imaging*. 2023;16:298–310. doi: 10.1016/j.jcmg.2022.07.013
23. Khalique OK, Hahn RT, Gada H, Nazif TM, Vahl TP, George I, Kalesan B, Forster M, Williams MB, Leon MB, et al. Quantity and location of aortic valve complex calcification predicts severity and location of paravalvular regurgitation and frequency of post-dilation after balloon-expandable transcatheter aortic valve replacement. *JACC Cardiovasc Interv*. 2014;7:885–894. doi: 10.1016/j.jcin.2014.03.007
24. Olsson M, Dalsgaard CJ, Haegerstrand A, Rosenqvist M, Ryden L, Nilsson J. Accumulation of T lymphocytes and expression of interleukin-2 receptors in nonrheumatic stenotic aortic valves. *J Am Coll Cardiol*. 1994;23:1162–1170. doi: 10.1016/0735-1097(94)90606-8
25. Yutzey KE, Demer LL, Body SC, Huggins GS, Towler DA, Giachelli CM, Hofmann-Bowman MA, Mortlock DP, Rogers MB, Sadeghi MM, et al. Calcific aortic valve disease: a consensus summary from the alliance of investigators on calcific aortic valve disease. *Arterioscler Thromb Vasc Biol*. 2014;34:2387–2393. doi: 10.1161/ATVBAHA.114.302523
26. Nadir MA, Wei L, Elder DH, Libianto R, Lim TK, Pauriah M, Pringle SD, Doney AD, Choy AM, Struthers AD, et al. Impact of renin-angiotensin system blockade therapy on outcome in aortic stenosis. *J Am Coll Cardiol*. 2011;58:570–576. doi: 10.1016/j.jacc.2011.01.063
27. Rosenhek R, Rader F, Loho N, Gabriel H, Heger M, Klaar U, Schemper M, Binder T, Maurer G, Baumgartner H. Statins but not angiotensin-converting enzyme inhibitors delay progression of aortic stenosis. *Circulation*. 2004;110:1291–1295. doi: 10.1161/01.CIR.0000140723.15274.53
28. Rossebo AB, Pedersen TR, Boman K, Brudi P, Chambers JB, Egstrup K, Gerdts E, Gohlke-Barwolf C, Holme I, Kesaniemi YA, et al. Intensive lipid lowering with simvastatin and ezetimibe in aortic stenosis. *N Engl J Med*. 2008;359:1343–1356. doi: 10.1056/NEJMoa0804602
29. Biancari F, Juvonen T, Onorati F, Faggian G, Heikkinen J, Airaksinen J, Mariscalco G. Meta-analysis on the performance of the EuroSCORE II and the Society of Thoracic Surgeons scores in patients undergoing aortic valve replacement. *J Cardiothorac Vasc Anesth*. 2014;28:1533–1539. doi: 10.1053/j.jvca.2014.03.014
30. Taleb Bendiab T, Brusset A, Estagnasie P, Squara P, Nguyen LS. Performance of EuroSCORE II and Society of Thoracic Surgeons risk scores in elderly patients undergoing aortic valve replacement surgery. *Arch Cardiovasc Dis*. 2021;114:474–481. doi: 10.1016/j.acvd.2020.12.004
31. Sa MP, Jacquemyn X, Van den Eynde J, Tasoudis P, Erten O, Sicouri S, Macedo FY, Pasala T, Kaple R, Weymann A, et al. Impact of paravalvular leak on outcomes after transcatheter aortic valve implantation: meta-analysis of Kaplan-Meier-derived individual patient data. *Struct Heart*. 2023;7:100118. doi: 10.1016/j.shj.2022.100118
32. Sá MP, Jacquemyn X, Sun T, Van den Eynde J, Tasoudis P, Erten O, Sicouri S, Torregrossa G, Clavel M-A, Pibarot P, et al. Late outcomes of permanent pacemaker implantation after TAVR: meta-analysis of

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reconstructed time-to-event data. *J Soc Cardiovasc Angiograp Int*. 2022;1:100434. doi: 10.1016/j.jscai.2022.100434
33. Cholesterol Treatment Trialists C, Fulcher J, O'Connell R, Voysey M, Emberson J, Blackwell L, Mihaylova B, Simes J, Collins R, Kirby A, et al. Efficacy and safety of LDL-lowering therapy among men and women: meta-analysis of individual data from 174,000 participants in 27 randomised trials. *Lancet*. 2015;385:1397–1405. doi: 10.1016/S0140-6736(14)61368-4
34. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, Kuder JF, Wang H, Liu T, Wasserman SM, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. *N Engl J Med*. 2017;376:1713–1722. doi: 10.1056/NEJMoa1615664
35. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, Chapman MJ, De Backer GG, Delgado V, Ference BA, et al. 2019 ESC/EAS guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. *Eur Heart J*. 2020;41:111–188. doi: 10.1093/eurheartj/ehz455
36. Cowell SJ, Newby DE, Prescott RJ, Bloomfield P, Reid J, Northridge DB, Boon NA, Scottish Aortic S; Lipid Lowering Trial IoRI. A randomized trial of intensive lipid-lowering therapy in calcific aortic stenosis. *N Engl J Med*. 2005;352:2389–2397. doi: 10.1056/NEJMoa043876
37. Chan KL, Teo K, Dumesnil JG, Ni A, Tam J, Investigators A. Effect of lipid lowering with rosuvastatin on progression of aortic stenosis: results of the aortic stenosis progression observation: measuring effects of rosuvastatin (ASTRONOMER) trial. *Circulation*. 2010;121:306–314. doi: 10.1161/CIRCULATIONAHA.109.900027
38. Rohatgi A, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, Neeland IJ, Yuhanna IS, Rader DR, de Lemos JA, et al. HDL cholesterol efflux capacity and incident cardiovascular events. *N Engl J Med*. 2014;371:2383–2393. doi: 10.1056/NEJMoa1409065
39. Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, French BC, Phillips JA, Mucksavage ML, Wilensky RL, et al. Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. *N Engl J Med*. 2011;364:127–135. doi: 10.1056/NEJMoa1001689
40. Zimmer S, Grebe A, Bakke SS, Bode N, Halvorsen B, Ulas T, Skjelland M, De Nardo D, Labzin LI, Kerksiek A, et al. Cyclodextrin promotes atherosclerosis regression via macrophage reprogramming. *Sci Transl Med*. 2016;8:333ra350. doi: 10.1126/scitranslmed.aad6100
41. Bode N, Grebe A, Kerksiek A, Lutjohann D, Werner N, Nickenig G, Latz E, Zimmer S. Ursodeoxycholic acid impairs atherogenesis and promotes plaque regression by cholesterol crystal dissolution in mice. *Biochem Biophys Res Commun*. 2016;478:356–362. doi: 10.1016/j.bbrc.2016.07.047
42. Niepmann ST, Lenart M, Willemsen N, Duewell P, Luetjohann D, Latz E, Nickenig G, Zimmer S. Dissolving cholesterol crystals reduces aortic valve stenosis development in mice. *Eur Heart J*. 2020;41:ehaa946-3719. doi: 10.1093/ehjci/ehaa946.3719
43. Gould S, Scott RC. 2-Hydroxypropyl-beta-cyclodextrin (HP-beta-CD): a toxicology review. *Food Chem Toxicol*. 2005;43:1451–1459. doi: 10.1016/j.fct.2005.03.007
44. Loftsson T, Jarho P, Masson M, Jarvinen T. Cyclodextrins in drug delivery. *Expert Opin Drug Deliv*. 2005;2:335–351. doi: 10.1517/17425247.2.1.335
45. Otto CM, Nishimura RA, Bonow RO, Carabello BA, Erwin JP III, Gentile F, Jneid H, Krieger EV, Mack M, McLeod C, et al. 2020 ACC/AHA guideline for the management of patients with valvular heart disease: a report of the American College of Cardiology/American Heart Association joint committee on clinical practice guidelines. *Circulation*. 2021;143:e72–e227. doi: 10.1161/CIR.0000000000000923
46. Sa MP, Van den Eynde J, Malin JH, Torregrossa G, Sicouri S, Ramlawi B. Impact of left ventricle outflow tract calcification on the outcomes of transcatheter aortic valve implantation: a study-level meta-analysis. *J Card Surg*. 2022;37:1379–1390. doi: 10.1111/jocs.16306

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