The Associations of Aortic Valve Sclerosis, Aortic Annular Increased Reflectivity, and Mitral Annular Calcification with Subsequent Aortic Stenosis in Older Individuals: Findings from the Cardiovascular Health Study

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Background: Although aortic valve sclerosis (AVS) is well described as preceding aortic stenosis (AS), the associations of AS with antecedent mitral annular calcification (MAC) and aortic annular increased reflectivity (AAIR) have not been characterized. In a population-based prospective study, the authors evaluated whether MAC, AAIR, and AVS are associated with the risk for incident AS.

Methods: Among participants of the Cardiovascular Health Study free of AS at the 1994-1995 visit, the presence of MAC, AAIR, AVS, and the combination of all three was evaluated in 3,041 participants. Cox proportional-hazards regression was used to assess the association between the presence of calcification and the incidence of moderate or severe AS in three nested models adjusting for factors associated with atherosclerosis and inflammation both relevant to the pathogenesis of AS.

Results: Over a median follow-up period of 11.5 years (interquartile range, 6.7-17.0 years), 110 cases of incident moderate or severe AS were ascertained. Strong positive associations with incident moderate or severe AS were found for all calcification sites after adjustment for the main model covariates: AAIR (hazard ratio [HR], 2.90; 95% CI, 1.95-4.32; $P < .0005$), AVS (HR, 2.20; 95% CI, 1.44-3.37; $P < .0005$), MAC (HR, 1.67; 95% CI, 1.14-2.45; $P = .008$), and the combination of all three (HR, 2.50; 95% CI, 1.65-3.78; $P < .0005$). In a secondary analysis, the risk for AS increased with the number of sites at which calcification was present.

Conclusions: In a large cohort of community-dwelling elderly individuals, there were strong associations between each of AAIR, AVS, MAC, and the combination of the three and incident moderate or severe AS. The novel finding that AAIR had a particularly strong association with incident AS, even after adjusting for other calcification sites, suggests its value in identifying individuals at risk for AS and potential inclusion in routine assessment by transthoracic echocardiography. (J Am Soc Echocardiogr 2023;36:41-9.)

Keywords: Mitral annular calcification, Aortic annular increased reflectivity, Aortic valve sclerosis, Incident aortic stenosis

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Abbreviations

AAIR = Aortic annular increased reflectivity

AS = Aortic stenosis

AVS = Aortic valve sclerosis

CHD = Coronary heart disease

CHS = Cardiovascular Health Study

CRP = C-reactive protein

CVD = cardiovascular disease

eGFR = Estimated glomerular filtration rate

HR = Hazard ratio

IL-6 = Interleukin-6

MAC = Mitral annular calcification

The prevalence of aortic valve sclerosis (AVS) increases with age such that approximately 30% of individuals >65 years of age have AVS, while 4% have overt aortic stenosis (AS).[1] Moreover, there is a marked increase in AS with advancing age such that the prevalence of AS increases almost eightfold in healthy individuals from 75-76 to 85-86 years old.[2]

The association of AVS with other sites of valvular calcification has been reported in observational studies, which suggest that mitral annular calcification (MAC) and AVS are associated with AS.[3,4] In a previous study of community-dwelling older adults in the Cardiovascular Health Study (CHS), AVS, MAC, and aortic annular increased reflectivity (AAIR), then called aortic annular calcification, were found in 54%, 42%, and 44% of participants, respectively, with combined MAC, AAIR, and AVS in 17% of individuals.[5]

Similarities between atherosclerosis and AS-like inflammation, lipid deposition, fibrosis, and calcification[6,7] have led to the hypothesis that atherosclerosis and inflammation may play a central role in the initial stages of aortic valve calcification and possibly initial stages of MAC and AAIR. However, previous studies[8,9] failed to find an association of high-sensitivity C-reactive protein (CRP) with AVS or incident AS.

Although AVS is known to progress to AS,[10,11] the associations of MAC and AAIR with incident AS, as separate exposures and as a combined exposure with AVS, have not been thoroughly studied. Recently, we completed the adjudication of incident AS in the CHS over a 25-year follow-up period, which gave us the opportunity to evaluate the relationships of clinically adjudicated AS with AVS, as well as components of fibrous skeleton calcification: AAIR and MAC.[12]

We postulated that the presence of AVS, AAIR, and MAC, both separately and combined, is associated with subsequent development of moderate or severe AS, independently of covariates known to be associated with incident AS.

METHODS

Participants

All individuals evaluated for this study were participants in the CHS, a prospective, community-based, epidemiologic observational study that was designed to assess cardiovascular risk factors and outcomes in elderly individuals. The design, rationale, and examination details of the CHS have been published elsewhere.[13] Of 5,888 participants (65-100 years of age) who were enrolled in the study, 5,201 were recruited in 1989 and 1990, and 687 predominantly Black participants were enrolled in 1992 and 1993. The study was approved by the institutional review board at each participating center, and informed consent was obtained from participants. Hospitalizations among participants were identified during semiannual contacts, and medical records for all hospitalizations were obtained. Cardiovascular events and deaths were adjudicated by a committee of physicians using standardized criteria. Echocardiographic examinations were performed on CHS participants at the 1989-1990 and 1994-1995 study examinations. The baseline for the present analyses was the 1994-1995 examination, during which echocardiographic assessment of cardiac calcification was performed.

Echocardiographic Identification of AVS, AAIR, and MAC

At the 1994-1995 examination, two-dimensional echocardiographic studies were recorded on videotape using a cardiac ultrasound machine (model SSH-160A; Toshiba) as previously described.[14] Echocardiographic studies were interpreted at a centralized core echocardiography laboratory (Georgetown University) by readers blinded to clinical information.

MAC was defined as an intense echo-producing structure located at the junction of the atrioventricular groove and posterior mitral leaflet on any of the parasternal long-axis, apical four-chamber, or parasternal short-axis views.

AAIR was defined as increased reflectivity of the aortic root at the insertion of the aortic cusps suggesting possible fibrosis and/or calcification. AVS was identified by aortic cusp thickening, normal aortic cusp excursion, and peak transaortic valve flow velocity < 2.0 m/sec.[10] Examples of these calcifications are illustrated in Supplemental Figures 1A to 1D. Intraobserver κ scores (116 observations) for MAC, AAIR, and AVS were 0.69, 0.60, and 0.80, respectively, indicating good agreement. For interobserver agreement (250 observations), the κ values for MAC, AAIR, and AVS were 0.36, 0.13, and 0.08, respectively.

Adjudication of AS

The end point adjudication methodology is described in detail elsewhere.[12] Briefly, prevalent and incident AS was ascertained on the 5,647 participants who underwent initial echocardiographic examinations in either 1989-1990 or 1994-1995 and had sufficient information available to classify AS.

The presence of AS on baseline CHS echocardiograms was based on restriction of leaflet opening of mild or greater severity or peak transaortic velocity ≥ 2.0 m/sec. Five screening methods were used to identify participants most likely to have AS during follow-up: (1) aortic valve procedure codes, (2) ICD diagnosis codes for AS and rheumatic aortic valve disease, (3) CHS heart failure hospitalization reviews, (4) CHS echocardiographic data, and (5) cause-of-death reviews. On the basis of these screening methods, 990 unique individuals were identified. To adjudicate AS, three physicians reviewed all available hospitalization records (and death records for those who were deceased) for these 990 participants through June 2014, including any echocardiography and procedure reports. The severity of AS during follow-up was ascertained from information in participant medical records, including echocardiographic and cardiac catheterization reports as available. Moderate AS was based on a peak transaortic velocity of 3.0 to 3.9 m/sec, a mean transaortic gradient of 25 to 39 mm Hg, or a calculated aortic valve area of 1.1 to 1.5 cm^2. Severe AS required a peak transaortic velocity ≥ 4.0 m/sec, a mean transaortic gradient ≥ 40 mm Hg, or a calculated aortic valve area ≤ 1.0 cm^2. AS was considered probable or definite if at least two of the three foregoing criteria were met. For the present

HIGHLIGHTS

- MAC, AAIR, and AVS are frequently found in elderly patients.
- These three calcifications are strongly associated with incident moderate/severe AS.
- The risk for AS increases with the number of calcification sites.
- Among these calcifications, AAIR has the strongest association with incident AS.
- AAIR should routinely be reported in clinical echocardiographic examinations.

analysis, the primary end point was probable or definite moderate or severe AS. We chose a primary outcome of moderate or severe AS because there were only a limited number of participants with severe AS. An aortic valve procedure for AS (surgical or transcatheter aortic valve replacement or percutaneous balloon valvuloplasty) was included in this primary outcome. Among those screened, the yield of probable or definite moderate or severe AS was as follows: procedure code, 84.8% (67 of 79); heart failure screen, 51.4% (91 of 177); CHS echocardiogram, 43.7% (118 of 270); and diagnostic code (ICD-9), 39.6% (263 of 664). The cause-of-death screen among those not identified above had a yield of 20.8% (five of 24). The yield on the basis of number of positive screens among the four main methods was as follows: one screen, 17.1% (112 of 656); two screens, 72.0% (134 of 186); three screens, 97.8% (45 of 46); and four screens, 100% (six of six). A sensitivity analysis showed that these screening methods achieved high capture for incident AS.[12]

Covariates

Covariates included age, sex, race (Black or not Black), enrollment year (1989-1990 or 1992-1993), body mass index, systolic blood pressure, antihypertensive medication use, diabetes, smoking (never, former, or current), estimated glomerular filtration rate (eGFR) by cystatin C, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, CRP, interleukin-6 (IL-6), coronary heart disease (CHD; myocardial infarction, angina pectoris, or coronary revascularization), stroke or transient ischemic attack, and peripheral artery disease. Demographic information was collected at enrollment. All other covariates were measured at the analysis baseline (1994-1995), except for height (used with weight measured at the 1994-1995 visit to calculate body mass index), eGFR, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, CRP, and IL-6, which were measured in 1992-1993.

Diabetes was defined as fasting glucose $\geq$ 126 mg/dL, nonfasting glucose $\geq$ 200 mg/dL, or use of diabetes medication (assessed via medication inventory).[15] CHD, stroke or transient ischemic attack, heart failure, and peripheral artery disease were based on standardized criteria and adjudicated by a committee of experts as previously described.[13] Cystatin C concentration was used to calculate eGFR, as a measure of renal function.[16] The biomarkers of inflammation, CRP and IL-6, were measured in serum stored at $-70\,^{\circ}C$ to $-80\,^{\circ}C$. As in previous studies, standardized approaches to collecting, aliquoting, and freezing samples were followed to minimize degradation.

Inclusion and Exclusion Criteria

Of the 5,888 participants enrolled in the CHS, 718 died before the 1994-1995 echocardiographic examination, 1,110 were alive but did not report for the 1994-1995 visit, and 31 reported for the visit but did not have echocardiograms. Overall, 4,029 were eligible for evaluation of MAC, AAIR, and AVS. The sample for the present study consisted of all participants evaluated for moderate or severe AS status diagnosed after the 1994-1995 echocardiographic examination. Participants were excluded from the analysis if they had undergone aortic valve replacement, were missing information on valve calcification on the echocardiogram, or had prior AS of any severity, resulting in 3,629 individuals. After omitting those with any missing calcification sites or main model covariates (described later), 3,041 participants were included in the analysis sample. After omitting those with missing exploratory model covariates (described below), 2,747 participants were included in the exploratory sample. The selection processes of the participants included in the analyses are presented in Figure 1.

Statistical Methods

For the 3,041 participants in the main analysis, we calculated descriptive statistics, summarizing continuous variables using means and SDs and summarizing categorical variables using counts and percentages. These descriptive statistics were calculated among those with MAC and those without MAC, regardless of other sites of calcification. This process of calculating descriptive statistics was repeated for those with and without AAIR and with and without AVS. Descriptive statistics were also calculated for those with MAC, AAIR, and AVS in combination and for the samples of those who had calcification at two, one, or zero sites. Finally, these descriptive statistics were calculated for those who did not have any of MAC, AAIR, or AVS.

Kaplan-Meier plots were generated to show the unadjusted association of moderate or severe AS with each calcification measure individually and the combination of MAC, AAIR, and AVS versus zero, one, or two calcification sites. Cox proportional-hazards regression was used to quantify the associations between MAC, AAIR, and AVS, individually and combined, and the AS outcome in the groups as defined above. In the combined model, those with MAC, AAIR, and AVS were compared with the group of those with zero, one, or two calcification sites. Time to event was calculated as the time between the 1994-1995 visit and the earliest of incident AS, death, loss to follow-up, or end of AS follow-up (June 2014). Tests of the

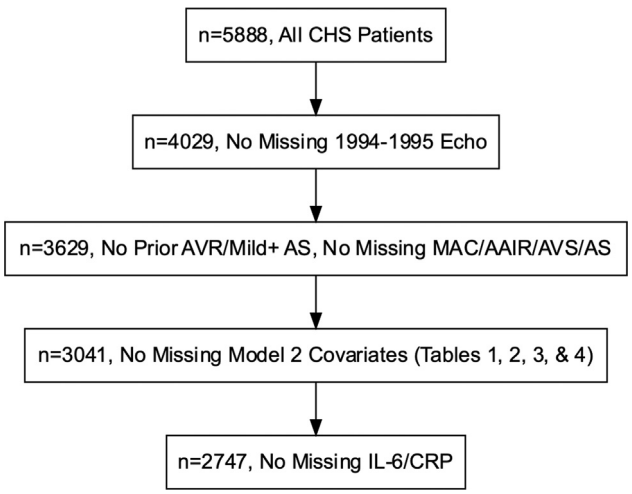

Figure 1 Flowchart of sample sizes used in the study.

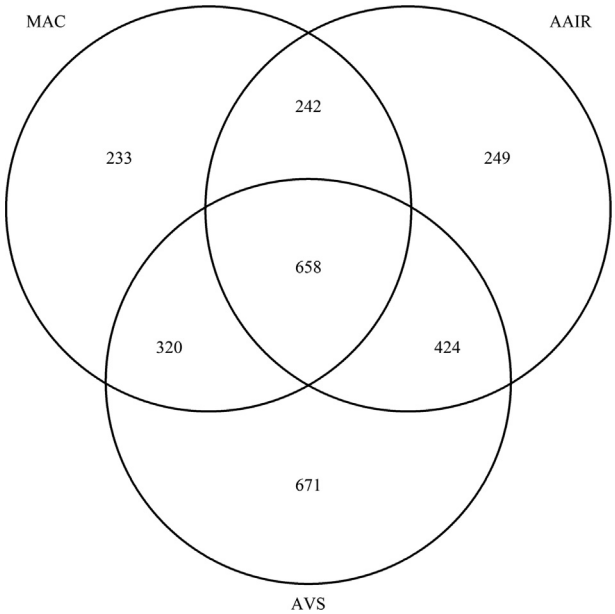

Figure 2 Venn diagram of calcification types (MAC, AAIR, and AVS) among the 3,629 individuals without prior AVR or mild or greater AS and without missing values for any of the 1994-1995 echocardiography, MAC, AAIR, AVS, or AS. Not illustrated are 832 individuals without any calcifications.

proportional-hazards assumption revealed no meaningful violations. Hazard ratio (HR) point estimates and CIs are presented, along with Wald *P* values. Sample sizes, numbers of events, and person-years for the time-to-event analyses are also presented.

Adjustment for covariates was done using three nested models. These were model 1, adjusted for age, sex, race, and enrollment year; model 2 (main model), model 1 plus body mass index, systolic blood pressure, antihypertensives, diabetes, smoking, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, history of CHD, history of stroke or transient ischemic attack, history of peripheral artery disease, and eGFR by cystatin C; and model 3 (exploratory model), model 2 plus CRP and IL-6. Primary analyses with models 1 and 2 used the sample of 3,041 individuals without any missing model 2 covariates, while exploratory analyses with model 3 used the sample of 2,747 individuals without any missing model 3 covariates.

After observing the results of the primary analyses detailed above, secondary analyses were conducted to assess whether the associations of each calcification site with incident AS were affected by adjusting for either or both additional calcification sites. All regression models in these secondary analyses adjusted for model 2 covariates and used the sample of 3,041 participants. We present HR point estimates, 95% CIs, and Wald *P* values, followed by pairwise linear hypothesis χ^2 test *P* values testing the null hypothesis of equality of calcification site HRs.

Additional exploratory analyses were conducted to evaluate the association of the number of calcification sites (AVS, AAIR, MAC) with AS. The group with no calcification sites was the reference category, while the presence of one, two, and three calcification sites were each treated as binary variables in the same model. A likelihood ratio test was performed to evaluate the significance of including variables for the number of calcification sites, comparing models with and without variables for one, two, and three calcification sites. Finally, we assessed the association of calcification with incident AS restricted to cases classified as severe. All regression models in these exploratory analyses used the sample of 3,041 participants.

Sensitivity analyses were conducted to test for effect modification of the association between the presence of calcification and AS by either sex or race by including the cross-product term into the model in the primary analyses.

Throughout this report, *P* values are not adjusted for multiple comparisons. Statistical analyses were conducted using R version 4.0.5 (R Foundation for Statistical Computing).

RESULTS

Among those with no missing model 2 covariates, 3,041 participants had adequate echocardiograms to evaluate MAC, AAIR, and AVS. MAC, AAIR, AVS, and their combination were identified in 40%, 43%, 57%, and 18% of included participants, respectively. There were substantial overlaps in calcification sites (Figure 2).

Demographic and clinical characteristics are shown in Table 1. There were no large differences among the calcification types. Compared with individual calcification sites, the prevalence rates of CHD, chronic kidney disease, and diabetes were slightly higher in the combined calcification exposure. Kaplan-Meier plots are shown for each calcification measure individually as well as MAC, AAIR, and AVS versus zero, one, or two calcification sites (Figure 3).

Association of Valve Calcification Exposures with Incident Moderate or Severe AS

The median follow-up time was 11.5 years. In all analyses of the sample of 3,041 individuals, there were 110 incident AS events and a total of 35,084 person-years. Strong positive associations with incident AS were found for all calcification sites in models 1 and 2, with minimal decreases in the HRs from model 1 to model 2. In each model, AAIR had the highest HR for incident AS, whereas MAC had the lowest. Adjusting for model 2 covariates, the risk for AS among those with AAIR was 2.90 times higher than in those without AAIR (95% CI, 1.95-4.32; $P < .0005$; Table 2). Adjusting for model 2 covariates, comparing participants with a calcification site versus those without that calcification site, MAC, AVS, and the combination of MAC, AAIR, and AVS had HRs of 1.67 (95% CI, 1.14-2.45; $P = .008$), 2.20 (95% CI, 1.44-3.37; $P < .0005$), and 2.50 (95% CI, 1.65-3.78; $P < .0005$), respectively (Table 2). Additional adjustment for CRP and IL-6 had virtually no effect on the HR estimates (results not shown).

In the secondary analysis, in which calcification sites were mutually adjusted for other calcification sites, AAIR was still strongly associated with AS after additionally adjusting for each of MAC (HR, 2.72; 95% CI, 1.80-4.11; $P < .0005$), AVS (HR, 2.61; 95% CI, 1.75-3.91; $P < .0005$), and MAC and AVS (HR, 2.48; 95% CI, 1.64-3.77; $P < .0005$; Table 3). The HR for MAC associated with AS was slightly decreased when adjusted for AVS (HR, 1.54; 95% CI, 1.05-2.26; $P = .028$), but more so when adjusted for AAIR (HR, 1.26; 95% CI, 0.84-1.87; $P = .261$) or both AAIR and AVS (HR, 1.20; 95% CI, 0.81-1.79; $P = .362$). Similarly, the HR for AVS decreased slightly with adjustment for MAC (HR, 2.09; 95% CI, 1.36-3.20; $P = .001$) but more so after adjustment by AAIR (HR, 1.84; 95% CI, 1.20-2.84; $P = .006$), and MAC and AAIR (HR, 1.82; 95% CI, 1.18-2.80; $P = .007$).

Additionally, the AAIR HR was significantly different from the MAC HR in the AAIR and MAC ($P = .019$) and AAIR, MAC, and

Table 1 Clinical characteristics and biomarker serum levels by calcification exposure, excluding those missing model 2 covariates or calcification information

Variable	MAC		AAIR		AVS		MAC, AAIR, and AVS		No calcification
	Yes (*n* = 1,217 [40%])	No (*n* = 1,824 [60%])	Yes (*n* = 1,312 [43%])	No (*n* = 1,729 [57%])	Yes (*n* = 1,740 [57%])	No (*n* = 1,301 [43%])	Yes (*n* = 542 [18%])	No (*n* = 2,499 [82%])	(*n* = 689 [23%])
Age, y	77 ± 5	76 ± 5	77 ± 5	75 ± 5	76 ± 5	76 ± 5	78 ± 5	76 ± 5	75 ± 5
Sex, male	467 (38)	742 (41)	528 (40)	681 (39)	744 (43)	465 (36)	210 (39)	999 (40)	244 (35)
Race, Black	176 (14)	327 (18)	201 (15)	302 (17)	304 (17)	199 (15)	85 (16)	418 (17)	124 (18)
Weight, kg	72 ± 14	73 ± 14	72 ± 14	73 ± 14	72 ± 14	73 ± 14	71 ± 14	73 ± 14	73 ± 15
Height,* cm	164 ± 10	165 ± 9	164 ± 10	165 ± 9	165 ± 10	164 ± 9	163 ± 10	165 ± 9	164 ± 9
BMI,† kg/m^2	27 ± 5	27 ± 4	27 ± 4	27 ± 5	27 ± 4	27 ± 4	27 ± 5	27 ± 4	27 ± 5
Systolic BP, mm Hg	135 ± 21	133 ± 20	135 ± 21	133 ± 20	135 ± 21	133 ± 20	137 ± 22	133 ± 20	133 ± 19
Enrollment year	138 (11)	262 (14)	169 (13)	231 (13)	239 (14)	161 (12)	71 (13)	329 (13)	101 (15)
HTN	736 (60)	996 (55)	773 (59)	959 (55)	1,025 (59)	707 (54)	333 (61)	1,399 (56)	368 (53)
Diabetes	237 (19)	308 (17)	242 (18)	303 (18)	332 (19)	213 (16)	118 (22)	427 (17)	117 (17)
Ever smoker	636 (52)	966 (53)	666 (51)	936 (54)	932 (54)	670 (51)	289 (53)	1,313 (53)	367 (53)
CHD	298 (24)	359 (20)	307 (23)	350 (20)	412 (24)	245 (19)	152 (28)	505 (20)	117 (17)
PAD	33 (3)	49 (3)	38 (3)	44 (3)	55 (3)	27 (2)	17 (3)	65 (3)	15 (2)
Stroke or TIA	114 (9)	109 (6)	100 (8)	123 (7)	136 (8)	87 (7)	54 (10)	169 (7)	41 (6)
CKD*	258 (21)	313 (17)	281 (21)	290 (17)	346 (20)	225 (17)	127 (23)	444 (18)	100 (15)
Antihypertensive medication	698 (57)	906 (50)	720 (55)	884 (51)	968 (56)	636 (49)	323 (60)	1,281 (51)	326 (47)
eGFR by CysC,* mL/min/1.73 m^2	74 ± 18	76 ± 18	74 ± 19	76 ± 17	75 ± 19	75 ± 17	73 ± 19	75 ± 18	76 ± 16
Total cholesterol,* mg/dL	205 ± 37	201 ± 36	203 ± 38	202 ± 36	203 ± 37	202 ± 37	206 ± 38	202 ± 37	201 ± 37
LDL-C,* mg/dL	124 ± 33	119 ± 33	122 ± 33	120 ± 33	122 ± 33	120 ± 33	125 ± 33	120 ± 33	118 ± 33
HDL-C,* mg/dL	53 ± 14	54 ± 15	53 ± 14	54 ± 15	53 ± 14	55 ± 15	53 ± 14	54 ± 14	55 ± 15
TG,* mg/dL	139 ± 63	136 ± 65	137 ± 65	137 ± 64	136 ± 64	138 ± 65	138 ± 64	137 ± 65	138 ± 67
CRP,* mg/L	5.26 ± 9.61	4.78 ± 7.52	4.88 ± 7.62	5.04 ± 8.98	5.02 ± 8.93	4.91 ± 7.7	5.22 ± 8.08	4.92 ± 8.5	4.95 ± 7.16
IL-6,* pg/mL	3.32 ± 2.12	3.14 ± 2.02	3.33 ± 2.1	3.12 ± 2.03	3.26 ± 2.05	3.14 ± 2.08	3.37 ± 2.01	3.17 ± 2.07	3.02 ± 1.93

Data are expressed as mean ± SD or as number (percentage).

BMI, Body mass index; *BP*, blood pressure; *CKD*, chronic kidney disease; *CysC*, cystatin C; *HDL-C*, high-density lipoprotein cholesterol; *HTN*, hypertension; *LDL-C*, low-density lipoprotein cholesterol; *PAD*, peripheral artery disease; *TG*, triglycerides; *TIA*, transient ischemic attack.

*Measured in 1992-1993.

†BMI calculated from weight measured in 1994-1995, height measured in 1992-1993, and all other covariates measured at enrollment or analysis baseline of 1994-1995.

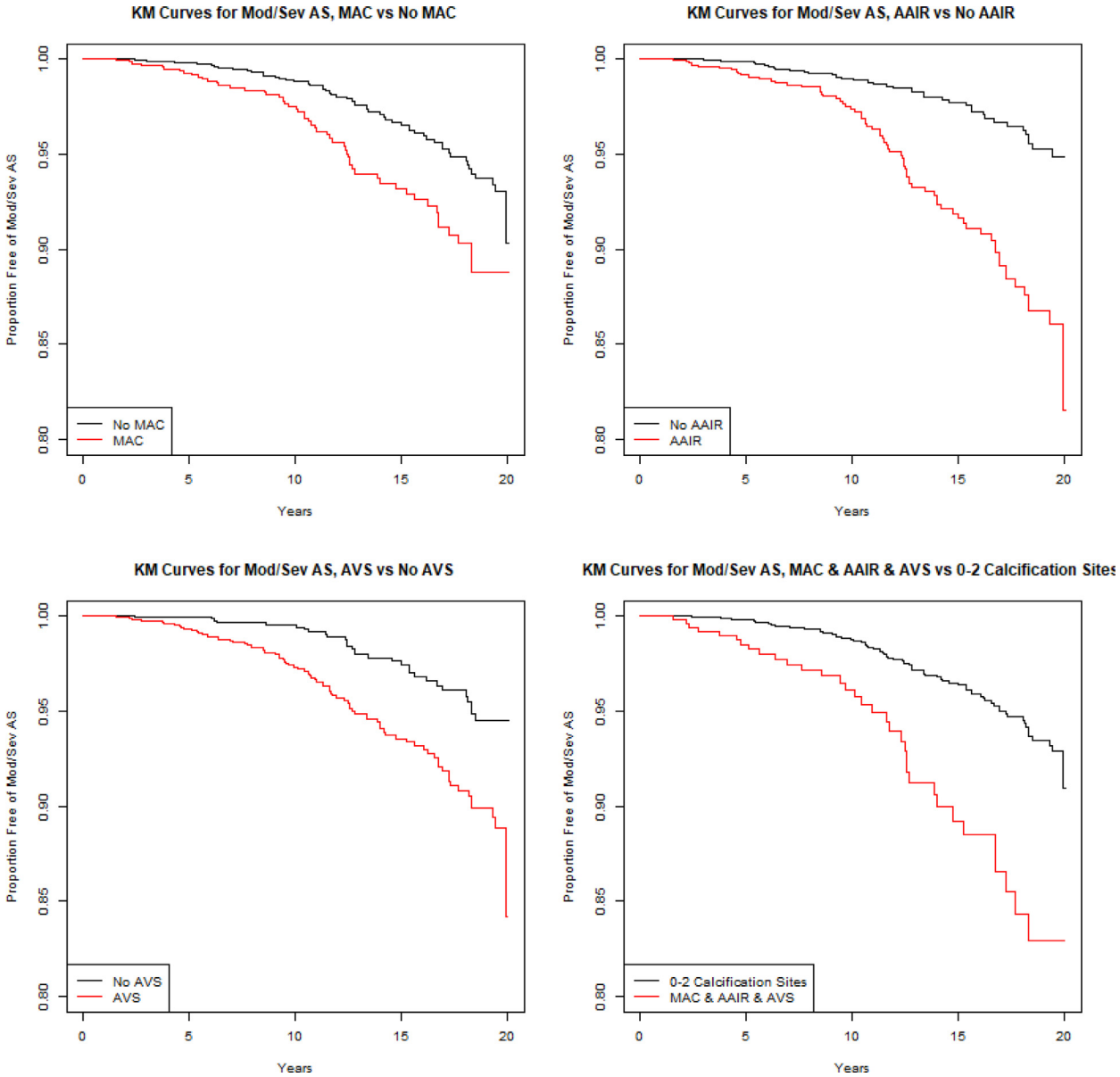

Figure 3 Kaplan-Meier (KM) curves for incident moderate (mod) or severe (sev) AS according to the cardiac calcification groups.

AVS ($P = .028$) models, whereas the AAIR HR was not significantly different from the AVS HR in either the AAIR and AVS ($P = .284$) or AAIR, MAC, AVS ($P = .341$) model. The MAC HR was not significantly different from the AVS HR in either the MAC and AVS ($P = .323$) or AAIR, MAC, and AVS ($P = .184$) model.

For the exploratory analyses comparing the number of calcification sites, among the 3,041 individuals without any missing MAC, AAIR, AVS, or model 2 covariates, 23% of participants did not have calcification at any site, 32% had one calcification site, 27% had two calcification sites, and 18% had MAC, AAIR, and AVS. Table 4 shows that the inclusion of the number of calcification sites in the model resulted in a significantly better fit to the data than the model without any calcification covariates, with a likelihood ratio test P value $< .0005$. Each calcification category was strongly positively associated with AS risk, and the HR was larger for those with more calcification sites.

We found no evidence of effect modification on the association between calcification and AS by either sex or race.

Of the 3,041 individuals included in our primary analyses, only 2% had incident severe AS. Compared with the analysis of moderate or severe AS, the HRs for the associations of calcification sites with severe AS were generally of similar magnitude, with the possible exception of the AVS HR, which appeared substantially higher for severe AS, albeit with wide CIs (HR, 5.66; 95% CI, 2.67-11.98; Supplemental Table 1).

DISCUSSION

The main finding of this study was that AAIR, MAC, AVS, and their combination were each significantly associated with incident moderate or severe AS over a median follow-up period of 11.5 years. Moreover, in our exploratory analyses, there was a progressive increase in the strength of the association of number of calcification sites with incident moderate or severe AS such that, in comparison with no

Table 2 Association of calcification by site with incident probable or definite moderate or severe AS

Model	Calcification	As HR (95% CI)	*P* value
Model 1	MAC	1.79 (1.23-2.62)	.002
	AAIR	2.97 (2.00-4.41)	<.0005
	AVS	2.32 (1.52-3.53)	<.0005
	MAC, AAIR, and AVS	2.76 (1.83-4.16)	<.0005
Model 2	MAC	1.67 (1.14-2.45)	.008
	AAIR	2.90 (1.95-4.32)	<.0005
	AVS	2.20 (1.44-3.37)	<.0005
	MAC, AAIR, and AVS	2.50 (1.65-3.78)	<.0005

For all models, *n* = 3,041, number of events = 110, and person-years = 35,084. Model 1 was adjusted for age, sex, race, and enrollment wave. Model 2 was adjusted for model 1 plus body mass index, systolic blood pressure, antihypertensives, diabetes, smoking, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, history of CHD (myocardial infarction, angina pectoris, or coronary revascularization), history of stroke or transient ischemic attack, history of peripheral artery disease, and eGFR by cystatin C.

calcification, the group with MAC, AAIR, and AVS had a markedly (sevenfold) greater risk for developing probable or definite moderate or severe AS. In an exploratory analysis of the association of cardiac calcification sites with incident severe AS found in 67 (2%) of participants, when compared with the association with moderate or severe AS, the only calcification category that appeared to have an outstanding higher HR was AVS, but the wide CIs dictate caution in the interpretation of this finding. Of particular interest is the novel finding that AAIR, rarely assessed in clinical practice, was strongly associated with incident AS, even after adjustment for risk factors, inflammation markers, and calcification at other sites. The increased aortic annular reflectivity could be the result of fibrosis, calcification, or of other processes. None of these hypotheses could be proved in the present study.

The poor interreader agreement did not prevent the determination of strong associations of calcification or AAIR with incident AS. Since the echocardiograms were obtained (1994-1995), there have been substantial improvements in echocardiographic equipment. It is likely that improved image quality and techniques such as biplane imaging available in current machines might improve the detection and quantitation of aortic annular reflectivity, as well as AVS and MAC. How this might affect the association of these echocardiographic findings with incident AS remains to be determined in future population studies.

Although the aortic root wall lacks valve interstitial cells, the aorta and aortic annulus do contain myofibroblasts as a related cell type that can also undergo differentiation into calcifying cells.[17] Moreover, studies using histopathologic techniques showed similarities between atherosclerosis in the vasculature and chronic fibrocalcific changes in the aortic valve and the mitral valve or annulus.[18-20]

It is likely that pathobiologic pathways (e.g., atherosclerosis, inflammation) are common to the development of AS, other ectopic calcifications (e.g., aortic and mitral annuli), and adverse outcomes in older individuals. Consistent with this is a previous study within the CHS, which showed that all three valve calcification sites are associated with all-cause and cardiovascular mortality.[5] Yet differences in risk factor profiles for AVC, AAIR, and MAC have been documented,[21,22] as has variation in their associations with outcomes.[23] As relates to the

Table 3 Association of calcification site with incident probable or definite moderate or severe AS after adjusting for additional calcification sites and model 2 covariates

Calcification site	Additional calcification covariates	Calcification site AS HR (95% CI)	Wald *P* value	Null hypothesis: MAC = AAIR, *P* value	Null hypothesis: MAC = AVS, *P* value	Null hypothesis: AAIR = AVS, *P* value
MAC	None	1.67 (1.14-2.45)	.008			
	AAIR	1.26 (0.84-1.87)	.261	.019		
	AVS	1.54 (1.05-2.26)	.028		.323	
	AAIR and AVS	1.20 (0.81-1.79)	.362	.028	.184	
AAIR	None	2.90 (1.95-4.32)	<.0005			
	MAC	2.72 (1.80-4.11)	<.0005	.019		
	AVS	2.61 (1.75-3.91)	<.0005			.284
	MAC and AVS	2.48 (1.64-3.77)	<.0005	.028		.341
AVS	None	2.20 (1.44-3.37)	<.0005			
	MAC	2.09 (1.36-3.20)	.001		.323	
	AAIR	1.84 (1.20-2.84)	.006			.284
	MAC and AAIR	1.82 (1.18-2.80)	.007		.184	.341

For all models, *n* = 3,041, number of events = 110, and person-years = 35,084. Wald *P* values correspond to the significance of calcification regression coefficients. Additionally, the null hypotheses of equality of calcification site coefficients are tested and *P* values presented. Model 1 was adjusted for age, sex, race, and enrollment wave. Model 2 was adjusted for model 1 covariates plus body mass index, systolic blood pressure, antihypertensives, diabetes, smoking, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, history of CHD (myocardial infarction, angina pectoris, or coronary revascularization), history of stroke or transient ischemic attack, history of peripheral artery disease, and eGFR by cystatin C.

Table 4 Association of number of calcification types with incident probable or definite moderate or severe AS

Model	Calcifications	AS HR (95% CI)	*P* value	Likelihood ratio test *P* value
Model 1	0 calcifications	1.0 (reference)		<.0005
	1 calcification	2.96 (1.36-6.45)	.006	
	2 calcifications	4.62 (2.15-9.94)	<.0005	
	MAC, AAIR, and AVS	7.97 (3.67-17.29)	<.0005	
Model 2	0 calcifications	1.0 (reference)		<.0005
	1 calcification	2.90 (1.33-6.32)	.008	
	2 calcifications	4.52 (2.09-9.76)	<.0005	
	MAC, AAIR, and AVS	7.16 (3.28-15.62)	<.0005	

For all models, n = 3,041, number of events = 110, and person-years = 35,084. Model 1 was adjusted for age, sex, race, and enrollment wave. Model 2 was adjusted for model 1 covariates plus body mass index, systolic blood pressure, antihypertensives, diabetes, smoking, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, history of CHD (myocardial infarction, angina pectoris, or coronary revascularization), history of stroke or transient ischemic attack, history of peripheral artery disease, and eGFR by cystatin C.

prominent association of AAIR with incident AS, it is possible that beyond shared underlying mechanisms, alteration in blood flow characteristics due to structural abnormality of the aortic annulus could increase biomechanical injury of the aortic valve leaflets, hastening development of significant AS.[24] However, the present study does not permit the evaluation of the causal mechanisms linking AAIR to incident AS.

In a retrospective study of 1,494 patients, the prevalence of MAC was threefold greater in individuals with AS than those without AS, and it was postulated that MAC may play a direct role in the pathogenesis of AS.[6]

In 381 individuals enrolled from the general population in the Stroke Prevention: Assessment of Risk in a Community study, atherosclerotic changes in proximity to the aortic valve (sinotubular debris and atherosclerosis of the ascending aorta) were strongly associated with AVS.[25] In another study of 1,242 individuals free of coronary artery disease who were evaluated using electron-beam computed tomography for the extent of calcium due to atherosclerosis in five distinct vascular beds and calcium in the aortic and mitral annuli, increased age and history of hypertension were the only traditional cardiovascular risk factors that were independently associated with prevalent aortic annular calcification and MAC. However, individuals with hypercholesterolemia, who were current or former smokers, or who had family histories of CHD had a significantly higher risk for aortic annular calcification but not MAC as those with calcium in the thoracic aorta.[26] Other studies have provided further evidence for the association between MAC and aortic annular calcification with CVD events.[27,28]

In concordance with prior studies, we found that participants with one or all sites of calcification were more likely to have histories of CHD, hypertension, and chronic kidney disease than those without those calcifications, suggesting that the presence of advanced atherosclerosis and hemodynamic alteration are associated with ectopic calcification on the valvular structures. Moreover, with an increased number of cardiac calcifications, the pace of calcium deposition on the aortic cusps could be accelerated, leading to clinically significant AS, which progresses over time.

Overall, we found AAIR in 43% of the participants, which concurs with the results of another large study.[26] Importantly, the association of AAIR with the risk for incident AS remained robust after adjusting for factors that were potentially associated with advanced atherosclerosis and ectopic calcification.

Strengths and Limitations

The prospective design and use of a population-based free-living cohort is a strength of the study, as is the adjudication of AS. However, because of the observational nature of the analyzed data, we could not infer mechanistic explanations for the strong association of AAIR and MAC calcification with AS. Although less clinically consequential than severe AS, moderate AS is an important outcome in its own right because it requires increased medical follow-up and, in the setting of a primary indication for coronary artery bypass graft surgery, is itself an indication for aortic valve replacement.[29] Nonetheless, the findings were consistent with our prespecified hypotheses that there were associations between incident AS and the three studied cardiac calcification sites. Despite the low interobserver agreement regarding AAIR, its association with incident moderate or severe AS remained robust in all statistical models. Nonetheless, we recognize that poor interobserver reliability as well as variability in echocardiographic acquisition may negatively affect clinical application of these (and other) echocardiographic findings. However, the echocardiographic studies that provided the data for this study were done using cardiac ultrasound technology available at that time. We believe that using modern echocardiographic equipment and enhanced examination techniques may improve the interobserver agreement.

Because of small numbers, we could not conduct a meaningful analysis of the risk for AS by calcification severity. The evaluation of ectopic cardiac calcification by echocardiography precludes accurate quantification of the calcium burden such as that provided by the Agatston method using cardiac computed tomography.[30] Despite this limitation, echocardiography can provide a qualitative or semiquantitative measure of calcification severity with important diagnostic and prognostic implications.

CONCLUSION

In a large cohort sampled from an elderly population, MAC, AAIR, AVS, and the combination of all three, were found in a significant number of subjects and had robust associations with significant AS, with AAIR having the strongest association. In exploratory analyses, the risk for AS increased with the number of calcification sites. AAIR retained a strong association with incident AS after additional

adjustment for each of MAC, AVS, and MAC and AVS. The particularly strong association of AAIR with incident AS suggests that this simple echocardiographic assessment might be used routinely in the clinical echocardiographic examination of older individuals. Research studies on AS prevention may consider including these echocardiographic measures of MAC and AAIR, as well as the more commonly used assessment of AVS. Further studies should be conducted to determine if the results of this study can be replicated.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found at https://doi.org/10.1016/j.echo.2022.08.013.

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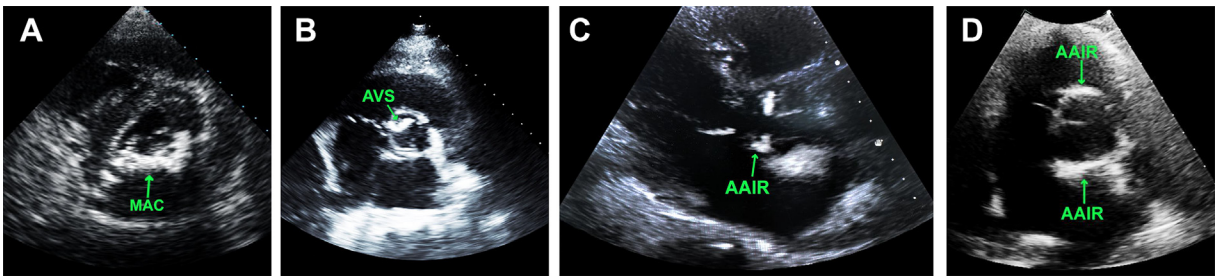
SUPPLEMENTARY DATA



Supplemental Figure 1 Examples of MAC, AVS, and AAIR. **(A)** Parasternal short-axis view of a transthoracic echocardiogram at the base of the left ventricle illustrating severe calcification (MAC) of the posterior aspect of the mitral annulus *(arrow)*. **(B)** Systolic frame of a parasternal short-axis view of a transthoracic echocardiogram at the aortic valve level showing calcification of the right coronary cusp (AVS; *arrow*). The peak velocity of the transaortic flow was 1.7 m/sec. **(C)** Diastolic frame of parasternal long-axis view showing increased brightness of the posterior aortic annulus *(arrow)*, suggesting tissue injury and/or calcification. **(D)** A parasternal short-axis view of the same image as in **C** illustrating increased echodensity (brightness) of the aortic annulus (AAIR; *arrow*) and the right and noncoronary cusps, suggesting AVS as well.

Supplemental Table 1 Association of calcification by site with severe AS

Model	Calcification	AS HR (95% CI)	*P* value
Model 1	MAC	1.41 (0.84-2.34)	.190
	AAIR	2.55 (1.51-4.29)	<.0005
	AVS	5.81 (2.76-12.25)	<.0005
	MAC, AAIR, and AVS	2.80 (1.62-4.87)	<.0005
Model 2	MAC	1.32 (0.79-2.20)	.295
	AAIR	2.51 (1.49-4.23)	.001
	AVS	5.66 (2.67-11.98)	<.0005
	MAC, AAIR, and AVS	2.49 (1.42-4.34)	.001