Title: Myocardial Blood Flow Reserve Is Impaired In Patients With Aortic Valve Calcification And Unobstructed Epicardial Coronary Arteries

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Abstract:

Background: Although calcific aortic valve disease (CAVD) is associated with coronary atherosclerosis, it is not known whether early CAVD is associated with coronary microcirculatory dysfunction (CMD). We sought to investigate the relationship between myocardial blood flow reserve (MBFR) - a measure of CMD, and early CAVD in the absence of obstructive epicardial coronary artery disease. We also determined whether this relationship was independent of coronary artery disease (CAD) and hs-CRP, a marker of systemic inflammation.

Methods: 183 patients with chest pain and unobstructed coronary arteries were studied. Aortic valve calcification score (AVCS), coronary total plaque length (TPL), and coronary calcium score were quantified from multislice CT. MBFR was assessed using vasodilator myocardial contrast echocardiography. Hs-CRP was measured from venous blood using a particle-enhanced immunoassay.

Results: Mean(±SD) participant age was 59.8(9.6) years. Mean AVCS was 68(258) AU, TPL was 15.6(22.2) mm, and median coronary calcification score was 43.5AU. Mean MBFR was 2.20(0.52). Mean hs-CRP was 2.52(3.86) mg/L. Multivariable linear regression modelling incorporating demographics, coronary plaque characteristics, MBFR, and inflammatory markers, demonstrated that age (β=0.05, 95%CI:0.02,0.08, P=0.007), hs-CRP (β=0.09, CI:0.02,0.16, P=0.010) and diabetes (β=1.03, CI:0.08,1.98, P=0.033), were positively associated with AVCS. MBFR (β=-0.87, CI:-1.44,-0.30, P=0.003), BMI (β=-0.11, CI:-0.21,-0.01, P=0.033), and LDL (β=-0.32, CI:-0.61,-0.03, P=0.029) were negatively associated with AVCS. TPL and coronary calcium score were not independently associated with AVCS when included in the regression model.

Conclusion: Coronary microvascular function as determined by measurement of myocardial blood flow reserve is independently associated with early CAVD. This effect is independent of the presence of coronary artery disease and also systemic inflammation.

1. Introduction:

The development of calcific aortic valve disease (CAVD) has traditionally been attributed to a passive, age-related degenerative phenomenon. However recent evidence suggests a more active mechanism. CT coronary angiographic studies have found an association between aortic valve calcification and the presence of coronary artery disease, which is an inflammatory process (1, 2). Epidemiologic studies have identified that risk factors for atherosclerosis - older age, male sex, hypercholesterolemia, hypertension, metabolic syndrome, and smoking, are also independently associated with the presence of CAVD (3). Histopathological studies have also observed the accumulation of atherosclerotic end products such as LDL, inflammatory cell infiltrates and microscopic calcification, within explanted or cadaveric CAVD specimens (4). In addition, more recent findings have shown that markers of inflammation such as hs-CRP, are independently associated with aortic valve calcification (5). In the light of these and other findings, several groups have suggested that CAVD and coronary atherosclerosis share common active pathophysiological mechanisms (6, 7).

The discovery of a link between both the early CAVD and coronary atherosclerosis has the potential to increase understanding of the disease, improve risk stratification and provide therapeutic targets. Coronary microvascular dysfunction (CMD) is a precursor to the development of coronary atherosclerosis (8). The presence of CMD has been shown to predict the future development of coronary artery disease, and also independently predict adverse cardiovascular and all-cause mortality. Therapies to improve CMD and outcome have had limited success (9-11). Whether or not CMD is independently associated with early CAVD is unknown. Previous studies have attempted to examine whether there is a direct link between CMD and CAVD. However, these have been limited to patients with established aortic stenosis (AS) which therefore represents late stage CAVD, or assessed CAVD with echocardiography rather than CT (12-14). Furthermore these studies either did not exclude or quantify whether coronary artery disease was also present (15). This is important because even the presence of mild non-obstructive coronary artery disease is known to be independently associated with CMD and therefore a confounding factor (16).

We sought to investigate the relationship between myocardial blood flow reserve (MBFR) - a measure of CMD, and early CAVD. We also determined whether this relationship was independent of coronary artery disease (CAD) and hs-CRP, a marker of systemic inflammation.

2. Methods:

2.1. Study population: This pre-determined study was part of another study investigating the relationship between chest pain typicality and its relationship to coronary microvascular function. This was a prospective, cross-sectional, observational study which recruited consecutive patients aged 30-80 years attending cardiology outpatient clinics (Jan 2011-March 2013) presenting with stable chest pain suggestive of myocardial ischaemia due to CAD and who were referred for diagnostic CT coronary angiography (CTA). Those with unobstructed coronary arteries underwent transthoracic echocardiography. Unobstructed CAD was defined as a quantitatively measured luminal diameter stenosis <50%. Patients were excluded if they had ≥50% luminal diameter narrowing, known ischemic heart disease, left ventricular hypertrophy, an ejection fraction <55%, or significant aortic valve disease. Specifically, those patients with a peak systolic transaortic valve velocity of >2m/s were excluded. Aortic valve calcification was quantified from the CTA study. Participants then underwent vasodilator myocardial contrast echocardiography (MCE) to assess myocardial blood flow reserve (MBFR). Informed consent was obtained for each patient. The study complied with the Declaration of Helsinki and was approved by the local research ethics committee (H0102/78).

2.2. CTA study: CTA imaging was performed using a 64-channel CT scanner (GE Lightspeed VCT, GE Medical Systems, USA). The mean heart rate during the scan was 64 beats/min. A low dose scout scan was performed to define anatomic landmarks for the contrast-enhanced study. Calcium scoring and helical scan data was performed on all patients using prospective ECG triggering at 75% of the R-R interval. Following this, a 20ml bolus of contrast (Niopram 370®) at 6mls/sec was injected and the timing of peak contrast enhancement in the aortic arch was used to determine the timing of scan acquisition. The contrast-enhanced scan used 80mls contrast injected at 6 ml/s, followed by a 50ml at 6mls/sec saline flush, during a single expiration breath hold.

The CTA scan parameters were: collimator 20mm, slice thickness 0.625mm; gantry rotation 350ms; helical acquisition using a pitch of 0.16; tube current 455-515 mA with ECG tube current modulation; tube voltage range 100-140kV; rotation time, 350ms. The estimated radiation dose per patient was 3.3mSv. Reconstructed CTA images were analysed on a dedicated 3-dimensional workstation (CardIQ Xpress, GE Medical Systems) with curved multiplanar reformation and short-axis cross sectional viewing techniques. We measured the amount of plaque present in the proximal, mid, and part of the distal sections, of the main vessel coronary artery tree. This decision was made because these segments have greater image quality regarding contrast:noise ratio, reduced observer variability, and contain the majority of clinically important plaque in the coronary tree (17, 18). This included the left main stem (segment 1), the first 80mm of the left anterior descending (divided equally into segments 2 and 3), the first 30mm of the circumflex (segment 4) and the first 80mm of the right coronary artery (segments 5 and 6).

2.3. Aortic Valve Calcification Score (AVCS): Amount of aortic valve calcification is correlated with the degree of aortic valve sclerosis applying multislice CT (19). For quantitative assessment of aortic valve calcification using CT with a detection threshold of 130H, the aortic valve Agatston score was derived. Calcification was attributed to the aortic valve if it was clearly part of the valve cusps. Supravalvular calcifications and calcifications of the coronary arteries including the ostia were removed by manual segmentation. The Agatston score was calculated by multiplying the lesion area by an attenuation factor derived from the maximal Hounsfield units within the area, as previously described (20). Figure 1 illustrates this analysis.

2.4. Coronary artery plaque burden: Total plaque length (TPL) was used as a surrogate measure of total atherosclerotic burden as described by Naya (21). Plaque length was measured as the distance (mm) from the proximal to the distal shoulder of each plaque. TPL was calculated for each patient by summing all the lengths for each subject. For each plaque, the degree of epicardial luminal narrowing was also assessed by quantifying the percentage diameter stenosis. This was calculated by dividing the minimal lumen diameter at each plaque by the nearest proximal normal artery diameter. The sum of all stenoses present divided by the number of stenosing plaques, determined the total mean stenosis per patient.

2.5. Coronary Calcium Score: 2.5mm slice thickness, non-overlapping images were reconstructed using filtered back projection and a standard algorithm (GE). The total calcium burden in the coronary arteries was manually measured by planimetry according to the scoring algorithm of Agatston (20).

2.6. MCE study: Patients underwent the MCE study having avoided all caffeine-containing products in the previous 24 hours. Patients withheld beta-blockers, nitrates and calcium antagonists the day before, and on the day of the test. MCE was performed using a commercial ultrasound machine iE33 (Philips Medical Systems) and SonoVue (Bracco Research SA) as the contrast agent given as a constant infusion. Real-time images were recorded within 3-4 minutes in the apical 4-, 2- and 3-chamber views with low-power settings at a mechanical index of 0.1. The focus was set at the mitral valve level. SonoVue was initially started at 60 mL/h through a peripheral vein cannula with the VueJect infusion syringe pump (Bracco Research, SA), which gently rotates and maintains the contrast agent in a suspension. Thereafter, the rate was set between 48 and 60 mL/h to maximize image quality with minimal attenuation. Once optimized, the machine settings were held constant throughout each participant study. Flash-impulse imaging at a high mechanical index (1.0) was performed to achieve complete myocardial bubble destruction, after which 10 end-systolic frames were recorded digitally in each apical view. After the resting images were acquired, dipyridamole was infused at 0.56 mg/kg over a 4-minute period. After an interval of 2 minutes, post-stress images were recorded within 3 to 4 minutes. This entire sequence took 14 minutes. Quantitative MCE analysis was performed offline using QLab V7.0 (Q-Laboratory, Philips Medical Systems) as previously described in detail (22). Briefly, quantitative assessment of myocardial perfusion was performed for 10 consecutive end-systolic frames after microbubble destruction. A region of interest was placed over the thickness of the myocardium. Plots of peak myocardial contrast intensity (representing myocardial blood volume A, dB) versus pulsing intervals (representing time) were automatically constructed to fit the monoexponential function conventional equation: $y=A\ (1 - e^{-\beta t})$. From these plots, the slope of the replenishment curve was determined (representing myocardial blood velocity β, dB/s). Figure 2 illustrates this analysis technique. The product of A and β yielded baseline MBF (dB^2/s) and post-dipyridamole MBF (stress MBF, dB^2/s), respectively. We calculated MBFR by the ratio of stress MBF (MBFs) to baseline MBF (MBFb). MBFR was calculated by dividing the MBFs by MBFb of the same segment. Basal segments were not included in the analysis because of contrast attenuation. The remaining 10 mid- and apical cardiac segments

were analyzed. A segment was not included in the analysis if there was artifact, inadequate microbubble destruction, attenuation, or a wide variation in contrast intensity to minimize errors.

2.7. Venous samples: Peripheral venous samples for hs-CRP were taken at rest. Fasting glucose and lipid profiles were from within the previous 3 months. All assays were performed in duplicate by a single observer, blinded to the demographic data. Hs-CRP was determined with a particle-enhanced immunoassay, which has a detection limit of 0.1mg/L and an inter-assay CV <10% (Roche Diagnostics, UK).

2.8. Statistical Analysis: Where appropriate, continuous variables were summarised using means, standard deviations and/or 95% confidence intervals. Ordinal and dichotomous variables were summarised using proportions or percentages. Indices of variance are bracketed after the respective value for central tendency. Normality was assessed using a Shapiro-Wilk test and if deemed necessary, heavily skewed data were transformed using a natural logarithmic transformation. General correlation was checked using a Pearson's correlation coefficient, and for categorical data, a Spearman's rank correlation (rho) test was employed. Statistical significance was tested using either a Student t-test for normally distributed continuous data or a Mann-Whitney U-test for non-normal continuous data. Ordinal and dichotomous data were tested using a Fischer exact test. Univariate regression was used initially to quantify the relationships between each of the explanatory variables and the main outcome variable MBFR against AVCS. Based on the results of the univariate regression, multivariable model building was performed using ordinary least-square regression and the resulting models were tested for coefficient (β) significance, model fit to data (adjusted R^2) and residual homoscedasticity and normality. If required, a leverage to squared residual plot was used to assess the effects of either high leverage or outlying values on the regression coefficients. A likelihood ratio (LR) test was employed to compare the fit of each successive iteration to the regression model. The level of significance was set at $P<0.05$. For CTA studies, 10 patients' scans (60 segments) were re-analysed by KN, and then again by two additional separate observers who were level 2 accredited or above (KG, RB). All analyses were performed using STATA^{TM} version 12.0.

3. Results:

Overall, our study included 183 participants whose baseline demographics are illustrated in Table 1. The mean age of participants was 59.8(±9.6) years of whom 52.5% were male with a mean BMI of 27.2(±3.8) kg/m^2. The mean hs-CRP was 2.52(±3.86) mg/L, with 75 (41%) participants having elevated hs-CRP levels (defined as >2mg/L).

3.1. Aortic valve analysis: In those with aortic valve calcification, the mean aortic valve calcification score (AVCS) was 68(±258) AU. In our cohort, the maximum AVCS was 2874 AU, and 72 (39%) had an AVCS of zero. Due to the marked right skew present in AVCS, values were normalised using a natural logarithmic transformation.

3.2. Coronary artery plaque morphology: From the 1096 segments available for analysis, 1016(92%) were interpretable. The total coronary tree length studied was 190mm plus the mean left main stem length (8mm+-7mm). Plaque was present in 113(62%) of patients. There were 384 plaques in total and 53(29%) of patients had either one or two plaques present. The mean stenosis per patient was 3.1(0-22)% of whom 138(76%) had a mean stenosis of <5%. Values for total plaque length (TPL) were right skewed with a median (IQRs) of 19.3 (8.9, 31.7) mm. The mean TPL was 15.6 mm, ranging from 0 to 132 mm. When present, the median coronary calcification score was 43.5(IQR 10.5-152) AU. Total coronary calcification score was also markedly right-skewed. Inter and intra-observer variability (kappa) for both aortic valve and coronary calcium scoring was 0.85 and 1.0, respectively. There were thirteen patients (7%) with an intramyocardial coronary course observed on CTA and all involved the left anterior descending coronary artery.

3.3. Myocardial blood flow: The mean MBFR was 2.20(±0.52), and 70 patients (38%) had an MBFR below 2.0. The intra- and inter-observer variability's for MBFR were 7.7% and 8.2%, respectively. The minimum number of analysable segments for baseline and stress was six for each, in keeping with previous published data (23).

Table 2 shows the results of univariate analysis performed on all demographic parameters in addition to total plaque length, coronary calcium score, hs-CRP and MBFR, in relation to aortic valve calcium score (AVCS). There were significant positive correlations between increasing age, total plaque length, and hs-CRP with AVCS. There was a significant negative correlation between MBFR, LDL and AVCS.

Multivariate model analysis incorporated all univariate parameters, each as independent explanatory variables, using stepwise deletion from a fully saturated model. Each iteration was checked using a likelihood ratio test against the fully saturated null model. No significant interaction terms were identified. The final regression equation is demonstrated in figure 3.

The tabulated values for regression coefficients (β), 95% confidence intervals and P-values for each β, are listed in Table 3. Age, diabetes, BMI, MBFR, LDL and hs-CRP are all independent predictors of AVCS. Coronary calcium score and TPL were included separately during the model evolution due to strong collinearity and neither were significant. As a result, the final model did not include either.

Figure 3 shows the regression model used to predict values for aortic valve calcification score. In the model, increasing age, presence of diabetes, and hs-CRP are independent positive predictors of ln(AVCS), whereas an increased MBFR, BMI, and LDL are negatively associated with ln(AVCS) (R^2=0.36).

4. Discussion:

Coronary microvascular function as determined by measurement of myocardial blood flow reserve is independently associated with early CAVD. This effect is independent of the presence of coronary artery disease and also systemic inflammation. Importantly, although coronary artery disease is initially associated with CAVD, when MBFR is included in the model, coronary artery disease is no longer predictive.

4.1. AVCS and microvascular function: Although previous studies have reported CMD in CAVD, they were conducted in patients with significant AS. For example, Rajappan assessed CMD in patients with moderate to severe AS with angiographically normal coronary arteries and used positron emission tomography to measure CMD (12). They found that parameters of AS severity such as aortic valve area and haemodynamic load were associated with CMD.

There is relatively limited data with regards to the relationship between early CAVD (without haemodynamically significant AS) and CMD. Only one study by Bozbas assessed the association between early CAVD and CMD. The group assessed CFR in patients with aortic valve calcification without significant AS. Aortic valve calcification was assessed using echocardiography (15). The authors found that mean CFR was 16% lower in the AVC group compared to control ($p<0.001$), and concluded that CMD is present even during early CAVD. Multivariate analysis found that the presence of aortic valve calcification was an independent predictor of CMD. However, although their patient cohort was asymptomatic, they pointed out that their study had not excluded obstructive coronary artery disease. This is important because even the presence of mild coronary artery disease is associated with CMD. For instance, Wang showed that increasing coronary calcium score in asymptomatic individuals is associated with reduced coronary perfusion reserve (16). In fact, endothelial dysfunction in the context of chest pain with unobstructed coronary arteries is a predictor of future coronary atherosclerosis (24).

Our study provides novel data for the following reasons. Firstly, early CAVD was diagnosed using CT, which is more accurate than echocardiography in the assessment of aortic valve calcification (25). For example, one group reported that CT had a greater sensitivity and detected 32% more patients than echocardiography in their elderly screening cohort (25). The Agatston score for aortic valve calcification provides a much broader range for quantitative scoring than the three point system used in Bozbas study. Finally, obstructive coronary artery disease was excluded definitively using CTA.

The role of microvascular dysfunction in relation to the development of CAVD is not yet clear. CMD has been shown to correlate closely with endothelial dysfunction (26) and peripheral endothelial dysfunction assessed using flow-mediated dilatation is also associated with aortic valve sclerosis. Therefore, when taken in conjunction with our results, this suggests a systemic process (27). The recent discovery that valve endothelial cells regulate the remodelling and integrity of the extracellular matrix within valve leaflets (28) may be important. It has been proposed that endothelial dysfunction allows inflammatory cytokine entry into valve leaflets which promotes mineralisation and ultimately leaflet calcification.

4.2. AVCS and inflammation: We found that the marker of systemic inflammation hs-CRP is independently associated with aortic valve calcification score and is consistent with other published data (29). Oxidative stress forms the initiating event of an inflammatory cascade which ultimately results in valvular calcification. Hs-CRP correlates strongly with oxidative stress and studies are underway regarding its use as a surveillance biomarker in the detection of atherosclerosis (5, 30). Furthermore, histopathology studies of explanted calcified aortic valves have found an abundance of leucocytes and macrophages (4). Our findings add to the literature in that CMD has a positive association with AVCS which is independent of and additive to the presence of systemic inflammation.

4.3. Other factors associated with early CAVD: Our results indicated that Increasing age and presence of diabetes were positively associated with AVCS. Neither of these findings are novel. The strong link between age and valvular calcification is well established from epidemiology studies (3). Diabetes is a pro-inflammatory condition which promotes macrophage deposition and consequent calcification (1).

Unexpectedly, in our analysis, increasing LDL was negatively associated with AVCS and is contrary to findings from previous studies (31, 32). Low-density lipoproteins have been implicated in the pathogenesis of CAVD. Extracellular lipid accumulation has been identified in explanted CAVD leaflets within the subendothelial layer with apolipoproteins, implying a plasma source (33). However, the role of LDL in the early stages of CAVD development is unknown especially given the failure of statin trials to slow AS progression (34). Importantly 36% of this patient cohort were on statin therapy prior to recruitment, and therefore a proportion of the study population may have a history of chronically elevated LDL. Furthermore, recent reports have suggested that lipid components other than LDL may be associated with AS progression and cardiovascular risk such as lipoprotein(a) (Lpa) and non-HDL, (35, 36).

Increasing BMI was negatively associated with AVCS in our analysis. This was unexpected as a high BMI is associated with both microvascular dysfunction and elevated systemic markers of inflammation, which promote calcification (37, 38). However, observational data showing increased survival rates in higher BMI

patients presenting with acute coronary syndromes: the so-called obesity paradox, draws consideration of whether higher BMI confers protection against certain disease processes (39).

4.4. Study Limitations: The study was conducted at a single centre in the UK which means referral bias may have affected our sample population. Furthermore our participants consisted of patients referred for the investigation of chest pain, which is not representative of the general population. However, the recruitment of this patient population enabled CT-based radiological investigation which otherwise would not have been ethically possible, and is consistent with previous studies (2, 15). Finally, the authors acknowledge that progression of early CAVD to haemodynamically significant AS is not absolute, and therefore no therapeutic implication can be derived from this study alone.

5. Conclusion:

Coronary microvascular function as determined by measurement of myocardial blood flow reserve is independently associated with early CAVD. This effect is independent of the presence of coronary artery disease and also systemic inflammation. Importantly, although coronary artery disease is initially associated with CAVD, when MBFR is included in the model, coronary artery disease is no longer predictive.

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Table 1: Baseline Demographics

		All patients (n=183)
Age in years; mean (SD)		59.8 (9.6)
Male sex (%)		96 (52.4)
Diabetes (%)		18 (10.0)
BMI (kg/m^2) (SD)		27.2 (3.8)
Smoking History (%)		
	Non smoker	100 (55)
	Ex-smoker	64 (35)
	Current smoker	19 (10)
Hypertensive (%)		71 (38.8)
Family history (%)		91 (49.7)
Statin use (%)		66 (36.0)

BMI: Body mass index

Table 2: Univariate regression modelling of demographic variables, lipid profile and inflammation markers, coronary plaque parameters and microvascular function against aortic valve calcium score.

	β	95% CI	P-value
Age (yrs)	+0.07	+0.03, +0.10	0.01
Male sex (Y/N)	+0.05	-0.61, +0.71	0.08
Diabetes (Y/N)	+1.08	-0.11, +2.06	0.07
BMI (kg/m^2)	-0.02	-0.12, +0.07	0.63
Smoking category (Y/N)	+0.31	-0.15, +0.77	0.19
Hypertension (Y/N)	+0.63	-0.02, +1.28	0.06
Family history (Y/N)	-0.36	-1.02, +0.30	0.29
Statin use (Y/N)	+0.63	-0.05, +1.30	0.07
LDL (mmol/l)	-0.45	-0.75, -0.15	0.003
Triglyceride (mmol/l)	+0.004	-0.33, +0.34	0.98
Total plaque length (mm)	+0.02	+0.01, +0.03	0.04
Coronary calcium score (AU)	+0.001	+0.001, +0.002	0.05
hs-CRP (mg/L)	+0.09	+0.01, +0.17	0.02
MBFR	-0.80	-1.41, -0.19	0.01

BMI: Body mass index, LDL: low-density lipoprotein, hs-CRP: high sensitivity C-reactive protein, MBFR: myocardial blood flow reserve

Table 3: Multivariate regression modelling for predicting aortic valve calcification score using age, presence of diabetes, BMI, myocardial blood flow reserve, LDL, and hs-CRP

Variable	β	95% CI	P-value
Age (yrs)	+0.05	+0.02, +0.08	0.007
Diabetes (Y/N)	+1.03	+0.08, +1.98	0.033
BMI (kg/m^2)	-0.11	-0.21, -0.01	0.033
MBFR	-0.87	-1.44, -0.30	0.003
LDL (mmol/l)	-0.32	-0.61, -0.03	0.029
hs-CRP (mg/L)	+0.09	+0.02, +0.16	0.010

BMI: Body mass index, MBFR: Myocardial blood flow reserve, LDL: low-density lipoproteins, hs-CRP: high sensitivity C-reactive protein

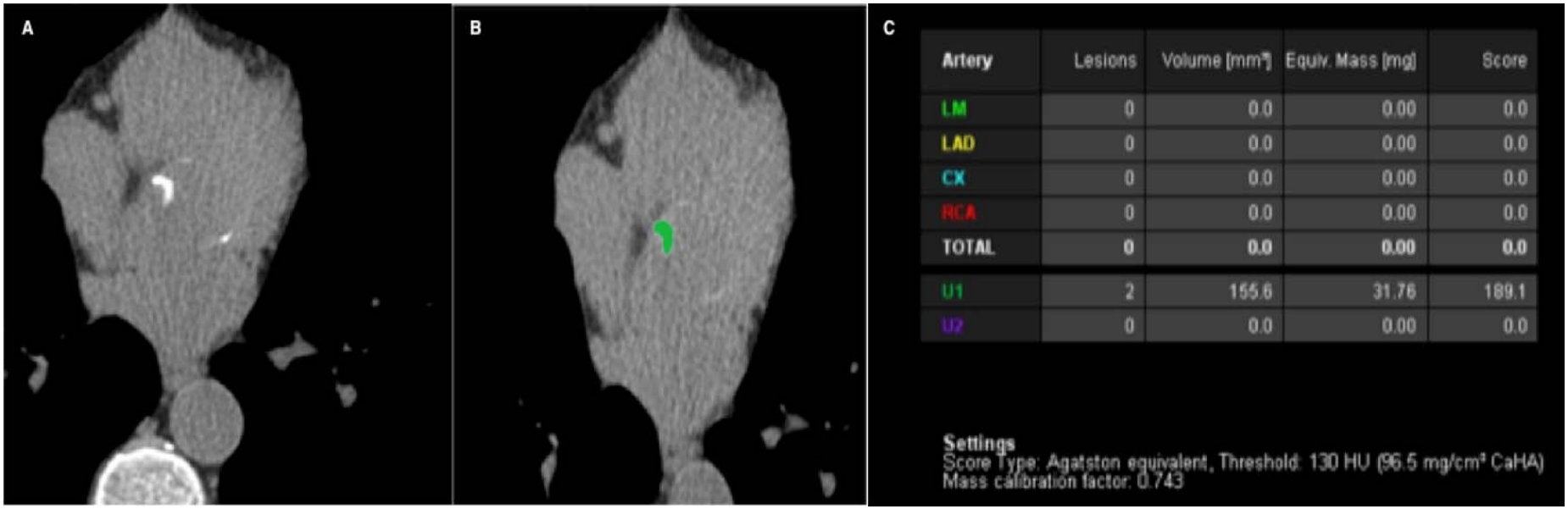
Figure Captions:

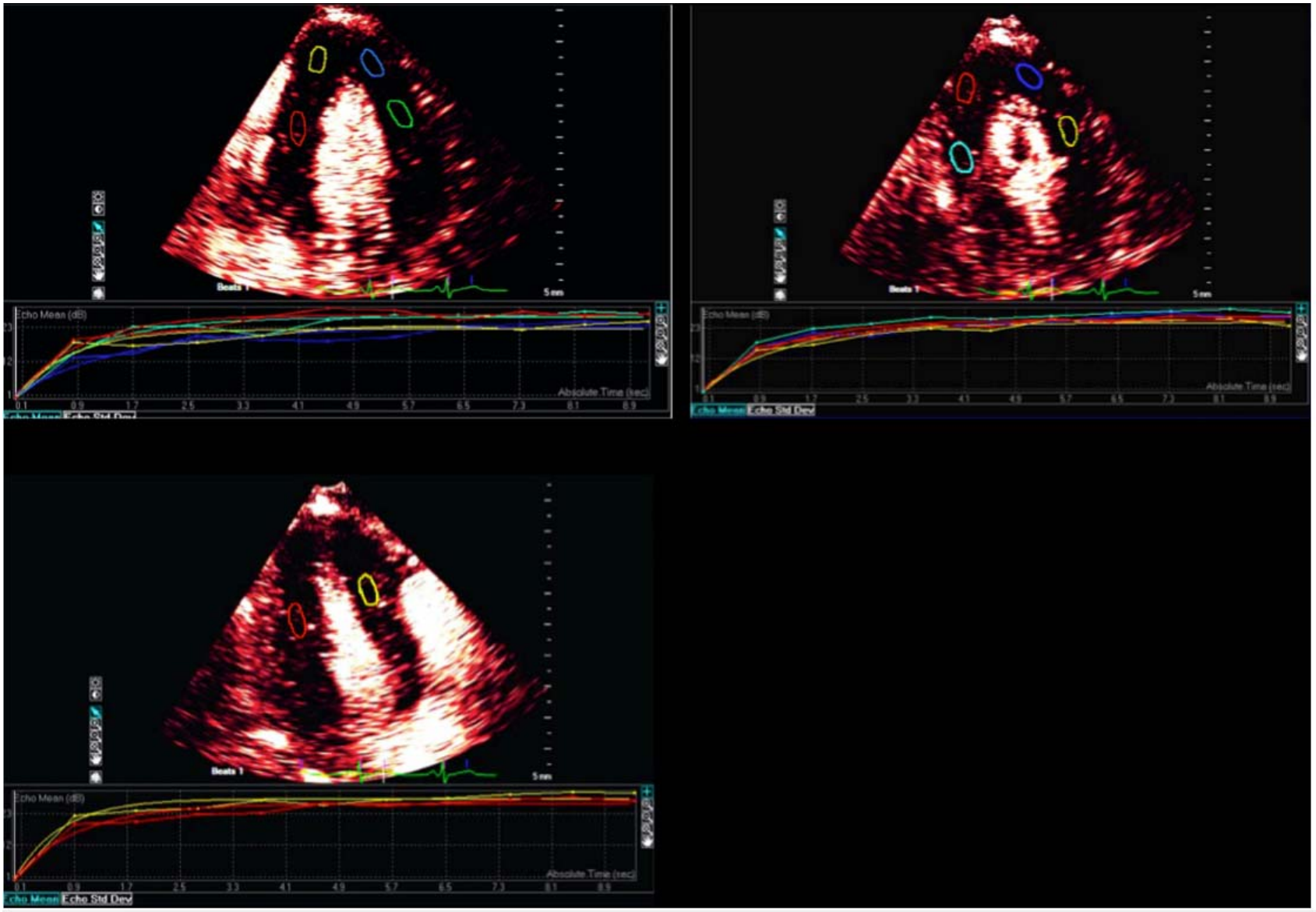
Figure 1. Example of aortic valve calcium scoring using ECG-gated coronary CT images. Calcification of aortic valve cusps detected on axial image (A). Imaging software detects regions within the aortic cusps with a density threshold of greater than 130HU to derive calcium volume (B). Aortic valve calcium score is then calculated - denoted in this case as 'U1' (C)

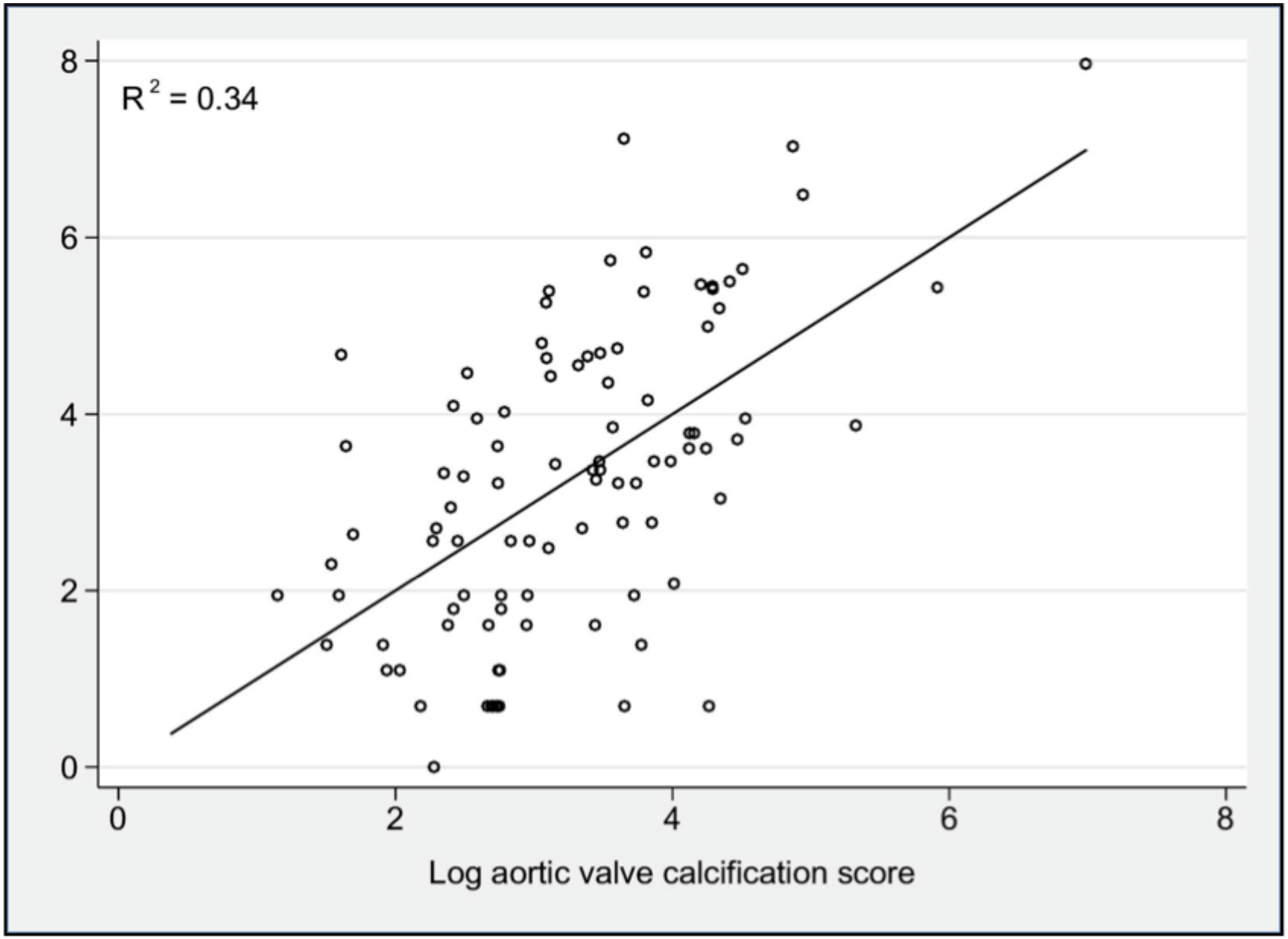
Figure 2. Model used for quantitative analysis of myocardial segments. (A) Apical 4 chamber, (B) Apical 2 chamber, (C) Apical 3 chamber.

Figure 3. Final model is described mathematically by ln(AVCS) = 5.83 + (0.05 x age) + (1.03 x diabetes) – (0.11 x BMI) – (0.87 x MBFR) – (0.32 x LDL) + (0.09 x hs-CRP)

Figure 1 (A, B and C) and Figure 2 and 3 below




Beats 1
5 mm
Echo Mean (dB)
Absolute Time (sec)
Echo Mean
Echo Std Dev


R^2 = 0.34
8
6
4
2
0
0
2
4
6
8
Log aortic valve calcification score