Symptom Onset in Aortic Stenosis – Relation to Sex Differences in Left Ventricular Remodelling

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Abstract
Objectives: To establish sex differences in remodelling and outcome in aortic stenosis (AS) and their associations with biomarkers of myocardial fibrosis.
Background: The remodelling response and timing of symptoms is highly variable in AS, and gender plays an important role.
Methods: 174 patients (133 male, mean age 66.2 ± 13.3 years) with asymptomatic moderate to severe AS underwent comprehensive stress cardiac magnetic resonance (CMR) imaging, trans-thoracic echocardiography (TTE) and biomarker analysis (MMP-2, 3, 7, 8 and 9, TIMP-1, 4, syndecan-1 and 4 and NT-proBNP), and were followed up at 6-monthly intervals. A primary endpoint was a composite of typical AS symptoms necessitating referral for AVR, cardiovascular death or major adverse cardiovascular events.
Results: For a similar severity of AS, male patients demonstrated higher indexed LV volumes and mass, more concentric remodelling (higher LV mass/volume), a trend to more late gadolinium enhancement (LGE) (present in 51.1% male *vs.* 34.1% female, p=0.057) and higher extra-cellular volume index than female patients (13.27 [11.5, 17.0] *vs.* 11.53 [10.5, 13.5] ml/m^2, p=0.017), with worse systolic and diastolic function and higher MMP-3 and syndecan-4 levels, whilst females had higher septal E/e'. Male sex was independently associated with indexed LV mass (β=13.32 (9.59-17.05), p<0.001). During median follow-up of 374 (IQR 351-498) days, a primary outcome, driven by spontaneous symptom onset, occurred in 21.8% of male and 43.9% of female patients (RR 0.50 (CI 0.31, 0.80), p=0.004). Measures of AS severity were associated with the primary outcome in both sexes, whereas NT-proBNP, MMP3 and mass/volume were only associated in males.
Conclusions: In AS, females tolerate pressure overload with less concentric remodelling and myocardial fibrosis but are more likely to develop symptoms. This may be related to higher wall stress and filling pressures in females.

Keywords: aortic stenosis, sex, remodelling, biomarkers

Abbreviations
AS: aortic stenosis
AVA(I): aortic valve area (index)
AVR: aortic valve replacement
CMR: cardiovascular magnetic resonance
ECV: extracellular volume
MPG: mean pressure gradient
PWV: pulse wave velocity
LGE: late gadolinium enhancement
LV: left ventricle
LVMI: left ventricular mass index
TTE: trans-thoracic echocardiogram
VAI: valvulo-arterial impedance

Introduction

Aortic stenosis (AS) is the commonest valve lesion requiring surgery in the developed world with increasing prevalence with aging populations (1). Symptomatic severe AS is a malignant condition for which International guidelines recommend aortic valve replacement (AVR). The exact mechanisms leading to symptoms are uncertain and patients may remain asymptomatic for many years. Considerable attention has focused on the role of left ventricular (LV) hypertrophy and other changes within the myocardium or "LV remodelling". This is initially thought to be adaptive, with increased wall thickness leading to normalisation of the wall stress and preservation of cardiac output (2). However, this eventually becomes maladaptive, leading to reduced myocardial perfusion (3,4), interstitial and replacement fibrosis (5,6) and impaired diastolic function (7) and systolic heart failure (8).

The remodelling response and the timing of symptom onset is highly variable. Various observational studies, mainly utilising trans-thoracic echocardiography (TTE) and cardiac catheterisation, have established differences in LV geometry and function between male and female patients with a similar degree of AS, with females demonstrating smaller, more concentrically thickened hearts, with 'supernormal' systolic function (9-11). Recent cardiac magnetic resonance (CMR) imaging studies have shown lower LV mass/volume in females (12,13).

Genome-wide association studies have also identified sex-dependent differences, with a greater profibrotic and inflammatory response to pressure overload in men, but supressed extra-cellular matrix remodelling and inflammatory gene pathways in female ventricles (14). Matrix metalloproteinases (MMP's) and their tissue inhibitors (TIMP's) have been implicated in pathological remodelling(15), progression to diastolic dysfunction(16) and heart failure in AS

(17). Additionally, Syndecan-1 and 4 are increasingly recognized to mediate pro-fibrotic signalling in cardiac fibroblasts (18). As sex differences in these markers have not previously been reported in AS, they were selected to reflect the likely differences in LV remodelling and myocardial fibrosis between sexes.

The aims of this study were to assess whether: differential LV remodelling in female and male patients with AS is associated with: 1. altered cardiac function and circulating biomarkers associated with myocardial fibrosis and 2. clinical outcomes in initially asymptomatic patients.

Methods

Subjects

Asymptomatic patients with moderate to severe AS were recruited as part of the 'PRognostic Importance of MIcrovascular Dysfunction in asymptomatic patients with AS' (PRIMID-AS) study(19,20). The national research ethics service approved the study and written informed consent was obtained from all participants.

Investigations

TTE

A comprehensive TTE was performed by an accredited sonographer according to International guidelines (21). All image analysis was conducted at the core lab by a single physiologist, using an Xcelera (Phillips, Best, The Netherlands) workstation, to assess AS severity, diastolic function, strain (using Speckle Tracking) and Valvulo-arterial impedance (VAI)(22). Blood pressure was measured on the same day at rest prior to performing the TTE.

CMR

Patients underwent comprehensive multi-parametric 3T CMR (including a stress and rest first-pass perfusion imaging, pre- and post-contrast T1 mapping and late gadolinium

enhancement (LGE) imaging after a total of 0.15 mmol/kg of gadolinium-based contrast agent) as previously described, at five sites within the UK(19). All image analysis was undertaken at the core lab by a single observer (AS), blinded to the patient data. Volumetric, T1 and LGE analysis was performed using *cvi42* version-5 (Circle Cardiovascular Imaging, Calgary, Canada). Papillary muscles were excluded from the myocardial mass analysis. The presence or absence of late-gadolinium enhancement (LGE) was visually determined by two experienced observers (GPM/AS), and classified as 'ischaemic' or non-ischaemic' distribution, and was quantified using >5SD above the mean signal intensity of normal myocardium(23). Extracellular volume (ECV) was calculated using Hct measured on the same day. We have previously shown excellent reproducibility of ECV calculation using dual-bolus contrast injection(24). Extracellular myocardial volume index (ECV× myocardial volume index) and myocyte volume index ([1-ECV] × myocardial volume index) were calculated(25). Quantitative perfusion analysis was performed using Q-mass version-7.1(4). Diogenes Feature Tracking software (TomTec Imaging Systems, Munich, Germany) was used for strain analysis(26). Pulse wave velocity (PWV) was calculated using Jim (Version 6, Xinapse systems, UK). VAI was also calculated using CMR-derived stroke volume (LVEDV-LVESV).

Plasma Biomarkers

Blood samples were collected in EDTA tubes and centrifuged within 4 hours at 2000g for 20 minutes. Plasma was then drawn off and stored at -80°C. Biomarker analysis was performed in a batch with a Luminex® bead-based multiplex assay(27), using antibodies from R&D Systems (Minneapolis, MN, USA). Colour-coded beads were pre-coated with a capture antibody for MMP-2, 3, 7, 8, 9, 12, TIMP-1 and 4, Syndecan-1 and 4, and added to the wells containing the sample. R-Phycoerythrin (RPE) secondary antibodies were then incubated with the samples.

After washing, the beads were read on a Luminex Bio-Plex 3D Reader (Bio-Rad, Hercules, CA, USA) (see Supplemental document). NT-proBNP was analysed using our in-house non-competitive assay that employs the quantitative sandwich enzyme immunoassay technique.

Follow-up and primary endpoint

Patients were followed up at 6-monthly intervals until a primary endpoint or end of study was reached. A primary endpoint was a composite of typical AS symptoms necessitating referral for AVR, cardiovascular death or major adverse cardiovascular events (hospitalisation with heart failure, chest pain, syncope or arrhythmia).

Statistical analysis

Baseline data was collected using electronic case-record forms, and blinded imaging data was sent to the Robertson Centre for Biostatistics, University of Glasgow, for unblinding and statistical analysis. Normally distributed data are expressed as mean±standard deviation. Non-parametric data are expressed as median[interquartile range]. Continuous variables were compared between male and female patients using independent t-tests or Mann-Whitney tests. The Chi-squared test or Fisher's exact test were used for categorical variables. Linear regression analysis was performed to look at correlations with LVMI and LV mass/volume. Univariate and multivariate associates of the primary outcome were determined using Cox proportional hazards regression and stepwise selection. Variables for the stepwise models were selected based on statistical significance ($p<0.05$) and clinical relevance (based on previously determined associations), avoiding co-linear variables.

Results

Demographic and echocardiographic data

174 subjects (133 male, 41 female) were recruited (table-1). Male patients were slightly older, with larger BSA. There was no difference in resting haemodynamics, incidence of most co-morbidities and common cardiovascular medication use. Men had slightly higher AVA but not when indexed to body surface area (AVAI) and had similar pressure gradients. . The septal E/e' was higher in females, as was the longitudinal peak early diastolic strain rate (PEDSR) (Speckle Tracking unanalysable in 52 patients: 41 male, 11 female). Moderate aortic regurgitation was present in five patients and none had more than mild mitral regurgitation. The demographic and remodelling data in the severe AS sub-group were similar (Supplemental Table 1).

CMR data

Male subjects had significantly higher indexed LV volumes and mass, more concentric remodelling (higher mass/volume ratio), and a lower systolic (LVEF, longitudinal and circumferential peak systolic strain) and diastolic (longitudinal and circumferential PEDSR) function than females(table-2, figure-1). Rest and stress MBF were significantly lower in males, with no difference in MPR, whilst PWV was significantly higher. The prevalence of LGE tended to be higher in male patients and extent of LGE was higher. There were 51 men and 11 women with non-infarct pattern LGE. There was no difference in native T1 but ECV was marginally higher in females but total extracellular myocardial volume and indexed extracellular myocardial volume were higher in males.

Plasma Markers

There was no significant difference in NT-proBNP levels between the sexes. Syndecan-4 and MMP-3 levels were higher in males. Whilst MMP-3 correlated with several CMR markers,

after adjusting for sex, these did not reach statistical significance. Syndecan-4 however, was associated with increased ventricular volumes (LVEDV, LVESV and RVEDV).

Associations with LVMI

Table 4 shows univariate and multivariate associations of LVMI. Male sex was significantly associated with LVMI for the overall population and remained on multivariate analysis (β=12.10 (7.55-16.64), p<0.001). AV Vmax and MPG were associated with LVMI for both sexes, AVA or AVAI were not (figure-2). Whilst longitudinal strain parameters were associated with LVMI in both sexes, circumferential parameters and ejection fraction were only significant in males. NT-proBNP, left atrial volume index and markers of focal and diffuse fibrosis were associated with LVMI in male patients only. Serum biomarkers were not associated with LVMI. The following variables were entered into a stepwise multivariate model: age, VAI(CMR), AV Vmax, PWV, diabetes and BMI. AV Vmax was independently associated with LVMI in both sexes, whilst VAI and BMI was also associated in males. Univariate and multivariate associations of LV mass/volume are shown in Supplemental Table 2.

Associations with primary outcome

During median follow-up of 374 (IQR 351-498) days, 18 (43.9%) females developed symptoms (1 of whom died shortly after symptom onset), compared to 29 (21.8%) endpoints in males (28 symptom onset and 1 sudden death) (RR 0.50 (CI 0.31, 0.80), p=0.004). There were no other MACE endpoints. Measures of AS severity were associated with the primary outcome in both sexes, whereas NT-proBNP, MMP3 and mass/volume were only associated in males (table-5). The following variables were entered into the stepwise multivariate model: $\log_{10}$(NT-proBNP), $\log_{10}$(MMP3), AV Vmax, VAI(CMR), LV mass/Volume, MPR, ECV and %LGE. On excluding ECV from the model, $\log_{10}$(NTpro-BNP) was significant for male patients instead.

Discussion

This is the first study to show that significant sex differences in CMR-detected fibrosis are associated with plasma biomarkers of LV remodelling and fibrosis, in asymptomatic patients with AS. Male patients demonstrated more concentric remodelling, cardiac dysfunction and fibrosis than females, with biomarkers associated with remodelling/fibrosis being significantly higher. Despite this, there was a higher incidence of symptom onset in females. This uncoupling of LV remodelling and symptoms between genders, that has not been recognized previously, is likely to be important in clinical management and merits further attention.

Remodelling

Our finding of more concentric LV remodelling (higher mass/volume) in males is contrary to previous TTE studies showing higher relative wall thickness in females (10,28). However, TTE measurements are based on a single basal slice, usually using M-mode, which has many assumptions about the shape and symmetry of the LV. CMR overcomes many of these limitations and is now regarded as the gold standard for quantitative LV assessment (29,30). Two CMR studies have also shown higher LVMI(12,13) and LV mass/volume (12) in male AS patients and Dweck *et al* showed that male sex was associated with LVMI (31).

Contrary to previous CMR studies(12,13), we saw a strong trend ($p=0.06$) towards less LGE in females. The likely reason for this apparent discrepancy is that in both previous studies, females had more severe AS and were also older in Dobson *et al*'s study. Our observation is unlikely to be spurious since females also demonstrated better function and lower levels of biomarkers associated with fibrosis. Previous histological studies have also confirmed that females have less myocardial fibrosis and lower collagen volume at the time of AVR (11).

Interestingly, ECV, which is widely regarded as a measure of diffuse interstitial fibrosis was *higher* in females than males. There are 2 likely explanations for this apparent discrepant finding. Firstly, ECV is more than just a measure of diffuse interstitial fibrosis, as it measures all the extracellular space, including the normal matrix supporting myocytes as well as intramyocardial blood vessels, and given that hematocrit tends to be lower in females, this may contribute to the higher ECV. The healthy ECV is ~25%(32), whilst interstitial fibrosis is often very low (~6.5%)(33), so in early disease ECV vastly overestimates diffuse fibrosis. Secondly, the normal range in healthy females is typically higher than males (32), so this may just represent normal values. Future studies assessing ECV should adjust for gender in their population. Consistent with females having less interstitial fibrosis was the finding of reduced total extra-cellular volume index.

Biomarkers

Syndecan-4, a cell surface proteoglycan that promotes collagen cross-linking, was associated with increased volumes, and may play an important role in LV remodelling. Increased levels of MMP3, which is a collagenase that breaks down collagen and basement membrane components, implies increased extracellular matrix turnover and remodelling, leading to collagen accumulation and fibrosis. This is the first study to report that MMP3 has sex-dependent expression differences in asymptomatic AS. Lower MMP3 in females has been found in other conditions including bacterial sepsis, stroke, and myocardial infarction (MI) (34,35) and it predicted LV dysfunction, remodelling and mortality after MI (36). Female sex steroids reduced collagen deposition 3 fold more than testosterone in human aortic smooth muscle cells, and testosterone increased gene and protein expression of MMP3 relative to both control and female sex steroids (37). In a mouse model of pressure overload, wild-type male mice developed

eccentric hypertrophy and more pronounced cardiac fibrosis, a difference that was abolished in oestrogen receptor-beta knockout mice (38). Given that MMP3 independently predicts the primary outcome in men only, and estradiol/progesterone is associated with reduced MMP3 expression, circulating MMP3 may be central to understanding the sex differences in phenotype in AS.

Collectively these data suggest that for a given degree of AS, females adapt with less concentric remodelling and less focal myocardial fibrosis, but still have a greater incidence of spontaneous symptom onset. There are several possible explanations for this seemingly counter-intuitive finding. Our main outcome measure was symptom onset, as we wanted to identify 'pre-symptomatic' patients who may benefit from prophylactic AVR, which is quite distinct from previous studies that have correlated fibrosis and remodelling with adverse prognosis, mainly mortality, including post-AVR. Females were likely to have higher wall stress, due to less adaptive concentric remodelling for a given pressure gradient (Law of Laplace), which is supported by higher resting myocardial blood flow and numerically higher NT-proBNP levels, which may lead to earlier symptoms. And although females had less focal fibrosis, they demonstrated higher LV filling pressure (higher septal E/e' associated with a similar degree of atrial remodelling) that may limit the ability to further compensate with increasing AS severity. Another possibility is that females, particularly the elderly, tend to be less physically active (39) and there may be subjective differences in the interpretation and acknowledgement of symptoms. If symptom onset leads to earlier intervention, in combination with less irreversible fibrosis(40,41), this may also explain the better post-operative long-term survival in some female subgroups(42-44).

Limitations

The number of female participants, and hence the number of endpoints reached, were relatively low, leading to limitations in statistical interpretation, particularly in multivariate analysis, and the findings should be confirmed in additional studies. Clinical outcome was, as expected, driven by symptom development and not hard clinical endpoints. However, symptoms heralds a rapid decline in prognosis and is an indication for AVR, so we feel this is a valid outcome measure. Although all fibrosis parameters tended to be higher in males than females, the difference for non-infarct LGE was not statistically significant. We measured circulating biomarkers associated with myocardial fibrosis but we cannot be certain that there is no contribution from other tissues.

Conclusions

Asymptomatic male patients with moderate to severe AS demonstrate more concentric LV remodelling, a trend to more myocardial fibrosis (and increased plasma markers of fibrosis) and cardiac dysfunction than females. However, there is dissociation between LV remodelling/fibrosis and symptom onset, which was more common in females, which requires further investigation.

Competency in medical knowledge

This work enhances our understanding of the gender differences in remodelling and symptom onset in AS, and their associations with biomarkers.

Translational outlook

This study highlights the need for further studies to establish the reasons for differential remodelling and symptom onset between sexes, and perhaps explore if sex-specific definitions of AS severity may have a role in the future.

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Figure Legends

Figure-1. An example of a male (top panel) and female (bottom panel) patient with similar degree of aortic stenosis.
The figure shows the end-diastolic frame of a short-axis cine (a,e), and-systolic still of a 3-chamber cine (b,f), native T1 map (c,g) and late-gadolinium enhancement image (d,h) with insertion point non-infarct pattern LGE in the male patient (arrows). (Male: AVAI=0.41cm2/m2, LVEDVI=122 ml/m2, LVMI=110g/m2, mass/vol=0.90; Female: AVAI=0.36cm2/m2, LVEDVI=71 ml/m2, LVMI=33g/m2, mass/vol=0.0.47)

Figure-2. Relationship between left ventricular mass index and markers of AS severity in male and female patients.
Positive correlation of LVMI with AV Vmax and MPG, and no correlation with AVAI.

Table 1. Demographic and echocardiographic data

	All (n=174)	**Male** (n=133)	**Female** (n=41)	**p-value**
Demographic data				
Age (years)	66.2 ± 13.34	67.3 ± 12.64	62.9 ± 15.08	0.066
BMI (kg/m2)	28.0 ± 4.15	28.0 ± 4.04	27.9 ± 4.53	0.826
BSA (m^2)	2.0 ± 0.21	2.0 ± 0.18	1.8 ± 0.17	<0.001*
HR (bpm)	70.3 ± 11.43	70.0 ± 11.11	71.2 ± 12.50	0.561
SBP (mmHg)	146.9 ± 21.09	148.2 ± 20.14	142.7 ± 23.70	0.146
DBP (mmHg)	77.2 ± 10.65	78.0 ± 10.40	74.3 ± 11.06	0.049*
Diabetes (n (%))	25 (14.4)	21 (15.8)	4 (9.8)	0.336
Hypertension (n (%))	93 (53.4)	70 (52.6)	23 (56.1)	0.697
Hyperlipidaemia (n (%))	92 (52.9)	78 (58.6)	14 (34.1)	0.015*
ACE-I/ARB (n (%))	77 (44.3)	58 (43.6)	19 (46.3)	0.758
Beta-blocker (n (%))	54 (31.0)	39 (29.3)	15 (36.6)	0.380
Statin	105 (60.3)	82 (61.7)	23 (56.1)	0.525
Echocardiography data				
AV Vmax (m/s)	3.86 ± 0.56	3.83 ± 0.54	3.97 ± 0.61	0.154
MPG (mmHg)	35.4 ± 12.49	34.5 ± 12.05	38.0 ± 13.66	0.121
AVAI (cm^2/m^2)	0.57 ± 0.14	0.58 ± 0.14	0.55 ± 0.15	0.206
AVA (cm^2)	1.12 ± 0.31	1.16 ± 0.30	0.96 ± 0.28	<0.001*
Severe AS (n(%))	123 (70.7)	93 (69.9)	30 (73.2)	0.845
E/A	0.88 ± 0.29	0.86 ± 0.26	0.96 ± 0.35	0.079
Septal E/e'	12.28 ± 4.86	11.71 ± 4.15	14.20 ± 6.43	0.029*
Lateral E/e'	9.88 ± 3.72	9.56 ± 3.43	10.94 ± 4.46	0.080
VAI (mmHg/ml/m^2)	3.96 ± 1.06	3.99 ± 1.08	3.86 ± 1.00	0.508
Longitudinal PSS (%)	-18.18 ± 2.76	-17.97 ± 2.79	-18.82 ± 2.58	0.140
Longitudinal PEDSR (1/s)	0.79 ± 0.21	0.76 ± 0.19	0.86 ± 0.25	0.024*

Abbreviations: BMI=body mass index, BSA=body surface area, HR=heart rate, SBP/DBP=systolic/diastolic blood pressure, ACE-I=angiotensin converting enzyme inhibitor, ARB=angiotensin II receptor blocker, AV Vmax=peak aortic jet velocity, MPG=mean pressure gradient, AVAI=aortic valve area indexed to BSA, AS=aortic stenosis, DPT=diastolic perfusion time, VAI=valvulo-arterial impedance, PSS=peak systolic strain, PEDSR=peak early diastolic strain rate

Table 2. CMR data for male and female patients

	Male (n=133)	**Female** (n=41)	**p-value**
LVEDVI (ml/m^2)	90.00 ± 18.67	79.74 ± 14.50	0.002*
LVESVI (ml/m^2)	39.97 ± 10.70	32.80 ± 8.49	<0.001*
LVSVI (ml/m^2)	50.04 ± 9.71	46.92 ± 7.48	0.061
LVEF (%)	55.9 ± 4.84	59.2 ± 4.49	<0.001*
LVMI (g/m^2)	60.54 ± 13.70	48.45 ± 9.74	<0.001*
LV mass/volume (g/ml)	0.68 ± 0.11	0.61 ± 0.11	0.001*
Myocyte volume index (ml/m^2)	42.28 [36.9. 48.2]	32.77 [30.6, 39.1]	<0.001*
Extracellular volume index (ml/m^2)	13.27 [11.5, 17.0]	11.53 [10.5, 13.5]	0.017*
LAVI (ml/m^2)	54.81 ± 14.43	55.46 ± 15.98	0.807
RVEDVI (ml/m^2)	91.26 ± 14.48	78.39 ± 13.07	<0.001*
VAI (mmHg/ml/m^2)	3.77 ± 0.81	3.95 ± 0.86	0.231
PWV (m/s)	8.76 ± 3.73	7.25 ± 2.71	0.005*
Stress MBF (ml/min/g)	2.09 ± 0.66	2.39 ± 0.80	0.020*
Rest MBF (ml/min/g)	0.93 ± 0.21	1.14 ± 0.36	0.002*
MPR	2.29 ± 0.70	2.18 ± 0.70	0.380
LGE present (n,%)	68 (51.1)	14 (34.1)	0.057
Non-infarct LGE (n,%)	51 (38.3)	11 (26.8)	0.178
LGE (g)	3.39 [10.6, 6.9]	0.95 [0.44, 2.7]	<0.001*
% LGE (%)	3.70 [1.03, 7.00]	1.60 [0.65, 4.30]	0.007*
Native T1 (ms)	1137.2 ± 71.06	1115.3 ± 62.73	0.139
ECV (%)	24.57 ± 2.54	25.64 ± 1.85	0.044*
Longitudinal PSS (%)	-17.85 ± 2.80	-20.52 ± 2.81	<0.001*
Longitudinal PEDSR (1/s)	1.04 ± 0.27	1.25 ± 0.26	0.030*
Circumferential PSS (%)	-27.64 ± 4.83	-29.56 ± 3.74	0.021*
Circumferential PEDSR (1/s)	1.60 ± 0.39	1.91 ± 0.36	<0.001*

Abbreviations: As in Table-1 and LVEDVI=left ventricular end-diastolic volume indexed to BSA, LVESVI=left ventricular end systolic volume indexed to BSA, LVSVI=left ventricular stroke volume indexed to BSA, LVEF=left ventricular ejection fraction, LVMI=left ventricular mass indexed to BSA, LAVI=left atrial volume indexed to BSA, RVEDVI=right ventricular end diastolic volume indexed to BSA, PWV=pulse wave velocity, MPR=myocardial perfusion reserve, MBF=myocardial blood flow, LGE=late gadolinium enhancement, ECV=extracellular volume

Table 3. Plasma biomarker data for male and female patients

	Male (n=33)	**Female** (n=41)	**p-value**
MMP 2 (ng/mL)	806 [503, 3615]	731 [469, 2230]	0.471
MMP 3 (ng/mL)	26.1 [15.3, 114.8]	17.6 [9.3, 46.8]	0.041*
MMP 7 (pg/mL)	823 [396, 1379]	735 [415, 1701]	0.638
MMP 8 (ng/mL)	2.28 [0.02, 5.66]	1.49 [0.02, 5.34]	0.659
MMP 9 (ng/mL)	93.9 [41.8, 214]	141 [59.6, 269]	0.429
MMP 12 (pg/mL)	67.8 [4.15, 117]	38.4 [4.15, 107]	0.842
TIMP 1 (ng/mL)	392 [269, 531]	344 [205, 519]	0.208
TIMP 4 (ng/mL)	3.8 [0.75, 379.7]	1.9 [0.51, 379.7]	0.116
Syndecan 1 (pg/mL)	185 [102, 319]	170 [73.1, 287]	0.464
Syndecan 4 (pg/mL)	213 [84.8, 449]	141 [1.07, 260]	0.043*
NT-proBNP (pg/mL)	53.8 [17.4, 144]	73.4 [22.7, 243]	0.165

Results expressed as median [IQR]. MMP=Matrix Metalloproteinase, TIMP=Tissue Inhibitor of Matrix Metalloproteinase, NT-proBNP=N terminal brain natriuretic peptide

Table 4. Univariate and multivariate associations with LVMI in male and female patients

Variable	Male		Female	
	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value
Age	-0.18 (-0.37, 0.00)	0.053	-0.21 (-0.40, -0.01)	**0.042**
Log(NTproBNP)	1.83 (0.55, 3.10)	**0.005**	0.07 (-1.65, 1.79)	0.932
AV Vmax	10.98 (7.03, 14.93)	**<0.001**	7.30 (2.72, 11.88)	**0.003**
MPG	0.48 (0.31, 0.66)	**<0.001**	0.32 (0.11, 0.52)	**0.004**
AVAI	0.45 (-16.9, 17.77)	0.959	12.99(-7.62, 33.60)	0.210
Septal E/e'	-0.18 (-0.76, 0.40)	0.540	-0.65 (-1.45, 0.14)	0.106
Lateral E/e'	-0.18 (-0.94, 0.59)	0.651	-0.19 (-1.23, 0.85)	0.714
VAI (CMR)	-4.91 (-7.72, -2.10)	**0.001**	-2.55 (-6.14, 1.05)	0.160
PWV	0.05 (-0.62, 0.71)	0.892	-0.84 (-2.00, 0.33)	0.154
LAVI	0.20 (0.04, 0.36)	**0.017**	0.01 (-0.19, 0.21)	0.932
LVEF	-0.83 (-1.30, -0.36)	**0.001**	-0.44 (-1.13, 0.25)	0.204
Rest MBF	-3.10 (-14.4, 8.20)	0.588	-8.23 (-15.3, -1.15)	**0.024**
Stress MBF	-3.28 (-6.97, 0.41)	0.081	-2.74 (-6.06, 0.59)	0.103
MPR	-2.83 (-6.30, 0.64)	0.109	-0.45 (-4.42, 3.51)	0.818
LGE presence	4.98 (0.34, 9.62)	**0.036**	-1.80 (-8.35, 4.74)	0.581
LGE %	0.56 (-0.06, 1.17)	0.075	-0.44 (-1.47, 0.60)	0.398
Native T1	0.06 (0.02, 0.10)	**0.003**	0.03 (-0.03, 0.10)	0.293
ECV	1.23 (0.05, 2.42)	**0.042**	-0.67 (-2.98, 1.64)	0.556
PSS-L (CMR)	1.21 (0.38, 2.03)	**0.004**	1.18 (0.13, 2.24)	**0.029**
PEDSR-L (CMR)	-18.2 (-26.6, -9.86)	**<0.001**	-14.7 (-26.0, -3.47)	**0.012**
PSS-C (CMR)	0.55 (0.06, 1.03)	**0.027**	0.05 (-0.80, 0.89)	0.913
PEDSR-C (CMR)	-10.4 (-16.2, -4.56)	**0.001**	-1.30 (-10.10, 7.48)	0.766
Log_{10} MMP3	2.08 (-1.36, 5.53)	0.234	-1.58 (-5.26, 2.09)	0.390
Log_{10} Syndecan4	1.53 (-0.30, 3.36)	0.100	0.46 (-1.98, 2.91)	0.702
Multivariate associations:-				
VAI (CMR)	-6.92 (-9.26, -4.59)	**<0.001**		
AV Vmax	14.35 (10.78, 17.92)	**<0.001**	7.31 (2.81, 11.81)	**0.003**
BMI	0.98 (0.51, 1.46)	**<0.001**		

Abbreviations: As Table-1 and 2**.** Stepwise multivariate analysis after entering the following variables: age, VAI (CMR), AV Vmax, PWV, diabetes and BMI.

Table 5. Univariate and multivariate associations with the primary outcome in male and female patients

Variable	Male		Female	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age	1.04 (1.00, 1.09)	0.072	1.01 (0.97, 1.04)	0.695
Log(NTproBNP)	1.46 (1.08, 1.98)	**0.015**	1.19 (0.88, 1.61)	0.264
AV Vmax	3.78 (1.86, 7.69)	**<0.001**	2.68 (1.21, 5.93)	**0.015**
MPG	1.06 (1.03, 1.09)	**<0.001**	1.04 (1.01, 1.08)	**0.010**
AVAI	0.00 (0.00, 0.17)	**0.003**	0.01 (0.00, 0.59)	**0.027**
VAI (CMR)	1.41 (0.86, 2.31)	0.175	1.52 (0.88, 2.65)	0.134
LVMI	1.01 (0.98, 1.04)	0.374	1.02 (0.97, 1.07)	0.511
LV mass / Volume	105.7 (4.01, 2784)	**0.005**	2.70 (0.03, 232.4)	0.662
LVEDVI	0.99 (0.96, 1.01)	0.309	1.00 (0.97, 1.04)	0.815
LAVI	1.03 (1.00, 1.06)	0.059	1.00 (0.98, 1.03)	0.793
LVEF	1.05 (0.96, 1.15)	0.251	0.94 (0.84, 1.05)	0.276
MPR	0.65 (0.34, 1.24)	0.189	0.51 (0.24, 1.11)	0.092
Stress MBF	0.51 (0.24, 1.09)	0.083	0.84 (0.45, 1.57)	0.574
Rest MBF	0.67 (0.09, 5.20)	0.704	2.57 (0.87, 7.61)	0.087
LGE presence	1.55 (0.66, 3.62)	0.316	0.77 (0.27, 2.18)	0.617
LGE %	1.01 (0.91, 1.12)	0.863	1.00 (0.85, 1.17)	1.000
Native T1	1.00 (0.99, 1.01)	0.714	1.00 (0.99, 1.01)	0.751
ECV	1.19 (0.99, 1.44)	0.069	0.91 (0.67, 1.23)	0.536
PSS-L	1.01 (0.87, 1.17)	0.931	1.15 (0.95, 1.39)	0.147
PEDSR-L	1.58 (0.34, 7.38)	0.563	0.85 (0.12, 5.81)	0.866
PSS-C	0.91 (0.83, 1.01)	0.066	1.00 (0.87, 1.14)	0.962
PEDSR-C	1.74 (0.61, 4.97)	0.303	0.95 (0.25, 3.62)	0.935
Log_{10} MMP3	1.84 (1.04, 3.28)	**0.037**	1.25 (0.69, 2.27)	0.459
Log_{10} Syndecan 4	0.97 (0.71, 1.34)	0.852	1.1 (0.75, 1.61)	0.617
Multivariate associations:-				
AV Vmax	5.29 (2.21, 12.67)	**<0.001**	3.09 (1.18, 8.06)	**0.022**
ECV	1.27 (1.03, 1.57)	**0.026**		
Log_{10} MMP 3	3.33 (1.54, 7.21)	**0.002**		

Abbreviations: As Table-1 and 2**.** Stepwise multivariate analysis after entering the following variables: log(NT-proBNP), AV Vmax, VAI (CMR), LV mass/Volume, MPR, ECV and %LGE. On excluding ECV from model, log(NTpro-BNP) and AV Vmax are independently associated with the primary outcome for male patients andAV Vmax remains for females.