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ORIGINAL RESEARCH ARTICLE

Galectin-3 as a candidate upstream biomarker for quantifying risks of myocardial ageing

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Abstract

Aims Galectin-3 (Gal-3) is implicated in the pathogenesis of heart failure and is also influenced by ageing. This study aims to determine the extent to which Gal-3 levels estimate odds of myocardial dysfunction in ageing cohorts, ‘upstream’ prior to clinical disease.

Methods and results Four hundred seventy-five asymptomatic subjects underwent simultaneous assessments of cardiovascular structure and function, with measurements of circulating Gal-3. Myocardial dysfunction was defined as impaired myocardial relaxation (ratio of peak velocity flow in early diastole E (m/s) to peak velocity flow in late diastole by atrial contraction A (m/s) <0.84) (mean E/A ratio 0.84 in the cohort). Of 475 subjects (mean age 68 ± 12 years, 231 women), 222 (47%) had myocardial dysfunction. Subjects with myocardial dysfunction were older (mean age 73 ± 5 vs. 64 ± 14 years, $P < 0.0001$), and more had hypertension (59 vs. 40%, $P < 0.0001$), dyslipidaemia (54 vs. 39%, $P = 0.001$), diabetes mellitus (25 vs. 14%, $P = 0.002$), higher body mass index (BMI) (24 vs. 23 kg/m^2, $P = 0.002$), and higher heart rate (76 vs. 71 b.p.m., $P = 0.0001$). Participants with impaired myocardial relaxation had lower peak velocity flow in early diastole E (0.6 ± 0.1 vs. 0.8 ± 0.2 m/s, $P < 0.0001$), higher peak velocity flow in late diastole by atrial contraction A (0.9 ± 0.1 vs. 0.7 ± 0.2 m/s, $P < 0.0001$), and higher mitral valve flow deceleration time (224.7 ± 43.2 vs. 204.8 ± 33.1 m/s, $P < 0.0001$). Participants with impaired myocardial relaxation had higher Gal-3 levels (17.2 ± 6.2 vs. 15.5 ± 4.1, $P = 0.0004$) but similar B-type natriuretic peptide (37 ± 4 vs. 34 ± 29, $P = 0.37$) and high-sensitivity troponin I (21 ± 72 vs. 11 ± 41, $P = 0.061$) levels and urine microalbumin-to-creatinine ratio (4.6 ± 8.1 vs. 4.2 ± 10.8, $P = 0.75$) compared with those without impaired myocardial relaxation. After multivariable adjustments, Gal-3 [odds ratio (OR) 1.05, 95% confidence interval (CI) 1.00–1.10, $P = 0.039$], age (OR 2.60, 95% CI 1.64–4.11, $P < 0.0001$), BMI (OR 2.16, 95% CI 1.44–3.23, $P < 0.0001$), and heart rate (OR 1.04, 95% CI 1.02–1.06, $P < 0.0001$) were associated with impaired myocardial relaxation. Adjusted ORs (95% CI) for myocardial dysfunction were 1.0 (ref), 1.62 (0.92–2.85), 1.92 (1.08–3.41), and 2.01 (1.11–3.66) across consecutive quartiles of Gal-3 after adjustment for age, BMI, risk factors, and heart rate.

Conclusions Among asymptomatic community-dwelling elderly adults, the highest quartile of Gal-3 was associated with two-fold increased odds of myocardial dysfunction compared with the lowest quartile of Gal-3. Gal-3 may have a role as an ‘upstream’ biomarker in estimating odds of myocardial ageing prior to clinical disease.

Keywords Galectin-3; Biomarker; Cardiovascular; Ageing

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Introduction

The ageing heart undergoes morphological alterations over time.[1–3] Ageing of the cardiovascular system is exemplified by alterations that include diastolic dysfunction, increased arterial stiffness, and impairments in endothelial functions.[2,4] These morphological and functional changes contribute to the prevalence of heart failure (particularly heart failure with preserved ejection fraction) in the ageing population.[5,6] The prevalence of heart failure in persons aged older than 75 years is approximately 8.4% compared with 0.7% in those aged 45–54 years.[7] Despite this huge burden of heart failure in older persons, elderly adults are poorly represented in clinical research.[8–10] To address this burden, tools that predict structural and functional alterations in the heart prior to incident cardiovascular disease are urgently needed to tackle cardiovascular disease risks in older persons.

Current literature and clinical practice emphasize investigations that use biomarkers to understand cardiovascular disease. However, there are hardly any investigations into candidate biomarkers that can represent processes of myocardial ageing that may predate incident cardiovascular disease.

Galectin-3 (Gal-3) is a β-galactoside-binding lectin that plays a role in inflammation, fibrosis, atherosclerosis, and heart failure.[11–14] High Gal-3 levels are associated with cardiovascular mortality and adverse outcomes,[15,16] while inhibition of Gal-3 has been found to prevent adverse cardiac remodelling.[17] Gal-3 levels increase with age and have been shown to be associated with cardiovascular risk factors[18] and ageing outcomes.[19] Given these properties, Gal-3 may represent a composite biomarker of age-associated cardiovascular ageing.

In this study, we hypothesize that Gal-3 levels may be associated with cardiovascular ageing among elderly adults, specifically defined by an early phase of myocardial ageing, representative of 'upstream' alterations prior to clinical disease. If our hypothesis is true, then Gal-3 levels may be used 'upstream' prior to cardiovascular development in ageing, to personalize an individual's risks of cardiovascular deterioration with age.

Methods

The subjects were recruited from the Cardiac Ageing Study (CAS),[20,21] a prospective study initiated in 2014 that examines characteristics and determinants of cardiovascular function in elderly adults. CAS participants were recruited from the local community and also from the prospective, population-based cohort, the Singapore Chinese Health Study.[22]

The study sample consisted of men and women who participated in the baseline CAS 2014 examination who had no self-reported history of physician-diagnosed cardiovascular disease (such as coronary heart disease and stroke) or cancer. The study complies with the Declaration of Helsinki. Written informed consent was obtained from participants upon enrolment. The SingHealth Centralised Institutional Review Board (2014/628/C) had approved the study protocol. All methods were performed in accordance with the relevant guidelines and regulations.

All participants were examined and interviewed on one study visit by trained study coordinators. Participants completed a standardized questionnaire that included medical history and coronary risk factors. Hypertension was defined by current use of antihypertensive drugs or physician-diagnosed hypertension. Diabetes mellitus was defined by current use of anti-diabetic agents or physician-diagnosed diabetes mellitus. Dyslipidaemia was defined by current use of lipid-lowering agents or physician-diagnosed dyslipidaemia. Smoking history was defined as ever smokers (former or current smoking) or never smokers. Body mass index was calculated as weight in kilograms divided by the square of height in metres. Sinus rhythm status was ascertained by resting electrocardiogram. Clinical data were obtained on the same day as assessment of echocardiography and serum collection.

Transthoracic echocardiography imaging

Echocardiography was performed using ALOKA α10 with a 3.5 MHz probe. In each subject, standard echocardiography, which included two-dimensional, M-mode, pulse Doppler and tissue Doppler imaging, was performed in the standard parasternal and apical (apical four-chamber, apical two-chamber, and apical long) views, and three cardiac cycles were recorded. The left ventricular ejection fraction, left atrial volume, and left atrial volume index were measured. The transmitral flow E and A waves with the sample volume position at the tip of the mitral valve leaflets from the apical four-chamber view were recorded by Doppler echocardiography. E/A ratio was computed as a ratio of peak velocity flow in early diastole E (m/s) to peak velocity flow in late diastole by atrial contraction A (m/s). Pulsed wave tissue Doppler imaging was performed with the sample volume at the septal and lateral annulus from the apical four-chamber view. The frame rate was between 80 and 100 frames per second. The tissue velocity patterns were recorded and expressed as E′ and A′. All measurements were measured by the same operator, and the measurements were averaged over three cardiac cycles and adjusted by the RR interval.

Biomarker measurements

Blood samples were collected on the day of echocardiography acquisition. Plasma levels of Gal-3 (ARCHITECT Galectin-3; produced by Fujirebio Diagnostics Inc for Abbott Laboratories), high-sensitivity troponin I (ARCHITECT STAT High Sensitive Troponin I; Abbott Laboratories), and B-type natriuretic peptide (BNP) (ARCHITECT BNP; produced by Fujirebio Diagnostics Inc for Abbott Laboratories) were measured on the Abbott ARCHITECT i2000SR analyser. Mid-stream urine samples were collected for analysis of random spot urine microalbumin-to-creatinine ratio. Urine microalbumin

(ARCHITECT Microalbumin; Abbott Laboratories) and urine creatinine (ARCHITECT Creatinine; Abbott Laboratories) were measured on the ARCHITECT cSystems.

Statistical methodology

We first examined bivariable association of subject clinical characteristics, cardiac function, and biomarkers with impaired myocardial relaxation. Impaired myocardial relaxation was defined as ratio of peak velocity flow in early diastole E (m/s) to peak velocity flow in late diastole by atrial contraction A (m/s) less than 0.84 (mean E/A ratio was 0.84 in our study sample).

Clinical characteristics, cardiac function, and biomarkers were compared between preserved and impaired myocardial relaxation using *t*-test or χ^2 test as appropriate. Continuous variables are reported as a mean with standard deviation.

Logistic regression models were constructed to assess the association of Gal-3 with impaired myocardial relaxation. The initial univariable logistic regression model examined the individual association with demographic variables and clinical covariates. Those variables associated in the univariable analysis with a $P < 0.05$ were candidate confounding factors associated with Gal-3. These candidates were then adjusted via multivariable logistic regression. We further fitted a logistic regression to estimate relative risks of impaired myocardial relaxation across Gal-3 quartiles controlled for potential confounding factors. To explore the shape of the association, we fitted restricted cubic splines with four knots at the 10th, 36.7th, 63.4th, and 90th, using the 12th percentile of Gal-3 as the reference.[23] Odds ratios (ORs) for impaired myocardial relaxation and the 95% confidence intervals (CIs) are shown in the graph. A Wald-type test for non-linearity yielded a *P*-value <0.0001, suggesting that a non-linear curve was the best fit for the data. The association between Gal-3 quartiles and E/A ratio is shown in age groups (<65, 65–75, and >75 years). The association between Gal-3 and other echocardiographic parameters is explored using linear regression (Supporting Information, *Table S1*).

All statistical analyses were performed using STATA 15 (College Station, TX, USA). For all analysis, a two-tailed *P*-value of <0.05 was considered significant.

Results

Baseline characteristics of the study population

A total of 475 participants (mean age 68 ± 12 years, 231 women) were included in the analysis. All completed clinical assessment, transthoracic echocardiography, and blood sampling on the same day.

The baseline characteristics of the study sample are shown in *Table 1*. There were 222 (47%) participants with impaired myocardial relaxation. Participants with impaired myocardial relaxation were older (mean age 73 ± 5 vs. 64 ± 14 years, $P < 0.0001$), and more had hypertension (59 vs. 40%, $P < 0.0001$), dyslipidaemia (41 vs. 39%, $P = 0.001$), diabetes mellitus (25 vs. 14%, $P = 0.002$), higher body mass index (24 ± 3 vs. 23 ± 4 kg/m^2, $P = 0.002$), higher systolic blood pressure (138 ± 20 vs. 148 ± 27 mmHg, $P < 0.0001$), and higher pulse rate (76 vs. 71 b.p.m., $P = 0.0001$). Participants with impaired myocardial relaxation had higher Gal-3 levels (17.2 ± 6.2 vs. 15.5 ± 4.1, $P = 0.0004$) but similar BNP (37 ± 4 vs. 34 ± 29, $P = 0.37$) and high-sensitivity troponin I (21 ± 72 vs. 11 ± 41, $P = 0.061$) levels and urine microalbumin-to-creatinine ratio (4.6 ± 8.1 vs. 4.2 ± 10.8, $P = 0.75$) compared with those with preserved myocardial relaxation.

Participants with impaired myocardial relaxation had greater interventricular septum thickness at end diastole (0.81 ± 0.1 vs. 0.78 ± 0.1 cm, $P = 0.013$), greater interventricular septum thickness at end systole (1.3 ± 0.2 vs. 1.2 ± 0.2 cm, $P = 0.0009$), greater left ventricular posterior wall at end diastole (0.8 ± 0.1 vs. 0.7 ± 0.1 cm, $P = 0.0002$), greater left ventricular posterior wall at end systole (1.5 ± 0.2 vs. 1.4 ± 0.2 cm, $P = 0.018$), greater left ventricular mass index (77.7 ± 32.3 vs. 71.5 ± 20.0 g/m^2, $P = 0.029$), greater isovolumic relaxation time (107.3 ± 19.8 vs. 95.2 ± 16.3 ms, $P < 0.0001$), lower peak velocity flow in early diastole E (0.6 ± 0.1 vs. 0.8 ± 0.2 m/s, $P < 0.0001$), higher peak velocity flow in late diastole by atrial contraction A (0.9 ± 0.1 vs. 0.7 ± 0.2 m/s, $P < 0.0001$), and higher mitral valve flow deceleration time (224.7 ± 43.2 vs. 204.8 ± 33.1 m/s, $P < 0.0001$). Notably, participants with impaired myocardial relaxation did not have other features of diastolic dysfunction: mean ratio of peak velocity flow in early diastole to peak early diastolic septal mitral annular velocity was 9.9 ± 2.8, mean peak early diastolic septal mitral annular velocity was 0.07 ± 0.02, mean pulmonary artery systolic pressure was 26.7 ± 7.5 mmHg, and mean left atrial volume index was 21.1 ± 7.1 (mL/m^2). All participants had preserved left ventricular systolic function. These results are presented in *Table 1*.

Galectin-3 and its relationship with age and myocardial function

Participants were divided into age subgroups (<65, 65–75, and >75 years) and quartiles of Gal-3 (*Figure 1*). In tandem with reductions in E/A ratio seen between the age groups, we observed increases in Gal-3 levels with age.

The univariable associations between Gal-3 and echocardiographic parameters are displayed in Supporting Information, *Table S1*. There was an association between Gal-3 and

Table 1 Baseline clinical characteristics, echocardiographic and biomarker data

	Preserved myocardial relaxation (n = 253)	Impaired myocardial relaxation (n = 222)	Total (n = 475)	P-value
Clinical variables				
Age (years)	64 (14.1)	73 (5.2)	67.8 (11.8)	<0.0001
Female gender	122 (48.2%)	109 (49.1%)	231 (48.6%)	0.85
Ever smoker	29 (17.9%)	52 (25.4%)	81 (22.1%)	0.087
Hypertension	101 (39.9%)	131 (59.0%)	232 (48.8%)	<0.0001
Dyslipidaemia	98 (38.7%)	120 (54.1%)	218 (45.9%)	0.001
Diabetes mellitus	35 (13.8%)	56 (25.2%)	91 (19.2%)	0.002
Body mass index (kg/m^2)	23 (3.6)	24.2 (3.2)	23.7 (3.4)	0.002
Systolic blood pressure (mmHg)	138 (19.7)	148 (26.5)	143 (23.6)	<0.0001
Diastolic blood pressure (mmHg)	74 (10.6)	74 (10.9)	74 (10.7)	0.90
Heart rate (b.p.m.)	71 (11.6)	76 (13.5)	73 (12.7)	0.0001
Echocardiographic markers				
IVSD (cm)	0.78 (0.1)	0.81 (0.1)	0.79 (0.1)	0.013
IVSS (cm)	1.2 (0.2)	1.3 (0.2)	1.2 (0.2)	0.0009
LVIDD (cm)	4.4 (0.6)	4.4 (0.6)	4.4 (0.6)	0.93
LVIDS (cm)	2.5 (0.4)	2.5 (0.5)	2.5 (0.5)	0.42
LVPWD (cm)	0.7 (0.1)	0.8 (0.1)	0.8 (0.1)	0.0002
LVPWS (cm)	1.4 (0.2)	1.5 (0.2)	1.4 (0.2)	0.018
LVOT (cm)	2.2 (2.0)	2.1 (0.2)	2.1 (1.5)	0.39
AO (cm)	2.9 (0.5)	3.1 (0.4)	3.0 (0.5)	<0.0001
LA (cm)	3.6 (0.5)	3.7 (0.6)	3.6 (0.5)	0.058
LVEF (%)	74.3 (6.9)	74.4 (7.9)	74.4 (7.4)	0.84
LVFS (%)	43.5 (6.3)	44.2 (7.8)	43.8 (7.1)	0.29
Left ventricular mass index (g/m^2)	71.5 (20.0)	77.7 (32.3)	74.8 (27.3)	0.029
Left atrial volume index (mL/m^2)	20.3 (7.5)	21.1 (7.1)	20.7 (7.3)	0.27
IVRT (ms)	95.2 (16.3)	107.3 (19.8)	100.4 (18.8)	<0.0001
Peak velocity flow in early diastole E (MV E peak) (m/s)	0.8 (0.2)	0.6 (0.1)	0.7 (0.2)	<0.0001
Peak velocity flow in late diastole by atrial contraction A (MV A peak) (m/s)	0.7 (0.2)	0.9 (0.1)	0.8 (0.2)	<0.0001
Mitral valve flow deceleration time (MV DT) (m/s)	204.8 (33.1)	224.7 (43.2)	214.1 (39.4)	<0.0001
PASP (mmHg)	26.2 (6.7)	26.7 (7.5)	26.4 (7.1)	0.51
Mitral A wave velocity duration (ms)	112.0 (13.2)	114.5 (15.2)	113.1 (14.2)	0.088
Peak systolic septal mitral annular velocity (septal S′) (m/s)	0.09 (0.05)	0.07 (0.01)	0.08 (0.04)	0.0023
Peak early diastolic septal mitral annular velocity (septal E′) (m/s)	0.09 (0.02)	0.07 (0.02)	0.08 (0.02)	<0.0001
Septal mitral annular velocity during atrial contraction (septal A′) (m/s)	0.2 (0.8)	0.1 (0.02)	0.1 (0.6)	0.35
Peak systolic lateral mitral annular velocity (m/s)	0.1 (0.03)	0.09 (0.03)	0.1 (0.03)	0.0012
Peak early diastolic lateral mitral annular velocity (m/s)	0.1 (0.03)	0.09 (0.02)	0.1 (0.03)	<0.0001
Lateral mitral annular velocity during atrial contraction (m/s)	0.117 (0.03)	0.125 (0.03)	0.120 (0.03)	0.0017
Ratio of peak velocity flow in early diastole E (MV E peak) to peak early diastolic septal mitral annular velocity (septal E′)	9.4 (3.1)	9.9 (2.8)	9.7 (3.0)	0.070
Biomarkers				
BNP (pg/mL)	34 (29.0)	37 (43.2)	35 (36.3)	0.37
High-sensitivity troponin I (ng/L)	10.6 (40.5)	20.6 (72.3)	15.3 (57.7)	0.061
Galectin-3 (ng/mL)	15.5 (4.1)	17.2 (6.2)	16.3 (5.3)	0.0004
Urine albumin-to-creatinine ratio (mg/mmol)	4.2 (10.8)	4.6 (8.1)	4.4 (9.4)	0.75

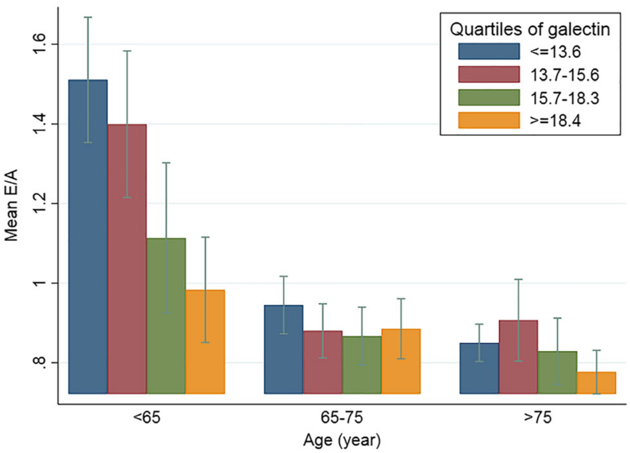
AO, aortic diameter; BNP, B-type natriuretic peptide; IVRT, isovolumic relaxation time; IVSD, interventricular septum thickness at end diastole; IVSS, interventricular septum thickness at end systole; LA, left atrium; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; LVIDD, left ventricular internal diameter end diastole; LVIDS, left ventricular internal diameter end systole; LVOT, left ventricular outflow tract; LVPWD, left ventricular posterior wall end diastole; LVPWS, left ventricular posterior wall end systole; PASP, pulmonary artery systolic pressure.

isovolumic relaxation time (β = 0.41, P = 0.032), peak velocity flow in late diastole by atrial contraction (β = 0.008, $P < 0.0001$), mitral valve flow deceleration time (β = 0.88, P = 0.01), pulmonary artery systolic pressure (β = 0.13, P = 0.038), pulmonary vein systolic velocity (β = 0.27, P = 0.012), pulmonary vein flow at atrial contraction (β = 0.14, P = 0.001), peak early diastolic septal mitral annular velocity (β = -0.001, $P < 0.0001$), peak early diastolic lateral mitral annular velocity ($\beta = -0.001$, $P < 0.0001$), and ratio of peak velocity flow in early diastole E to peak early diastolic septal mitral annular velocity (β = 0.07, P = 0.007).

Determinants of impaired myocardial relaxation

At the univariate level, Gal-3 levels (OR 1.09, 95% CI 1.04–1.14, $P < 0.0001$), age (OR 2.57, 95% CI 1.69–3.90, $P < 0.0001$), body mass index (OR 1.96, 95% CI 1.35–2.84, $P < 0.0001$), hypertension (OR 2.17, 95% CI 1.50–3.13,

Figure 1 Distribution of galectin-3 (in quartiles) with age and myocardial function. Participants were divided into age subgroups (<65, 65–75, and >75 years) and quartiles of galectin-3 levels. In each subgroup of age, E/A ratio decreased with increasing levels of galectin-3. Error bars denote standard errors. E/A, ratio of peak velocity flow in early diastole E (m/s) to peak velocity flow in late diastole by atrial contraction A (m/s).



$P < 0.0001$), dyslipidaemia (OR 1.86, 95% CI 1.29–2.68, P = 0.001), diabetes mellitus (OR 2.10, 95% CI 1.32–3.36, P = 0.002), and heart rate (OR 1.03, 95% CI 1.01–1.05, $P < 0.0001$) were associated with impaired myocardial relaxation. After multivariable adjustments, Gal-3 (OR 1.05, 95% CI 1.00–1.10, P = 0.039), age (OR 2.60, 95% CI 1.64–4.11, $P < 0.0001$), body mass index (OR 2.16, 95% CI 1.44–3.23, $P < 0.0001$), and heart rate (OR 1.04, 95% CI 1.02–1.06, $P < 0.0001$) remained independently associated with impaired myocardial relaxation. These results are presented in *Table 2*.

In a multivariable adjusted model, the highest quartile of Gal-3 was associated with two-fold higher odds of impaired myocardial relaxation compared with the lowest quartile (OR 2.01, 95% CI 1.11–3.66) (*Table 3*).

Data fitting using cubic splines revealed that the magnitude of the association between Gal-3 and impaired myocardial relaxation increased substantially as Gal-3 increased (*Figure 2*).

Table 3 Odds ratio (95% CI) for impaired myocardial relaxation by quartile of galectin-3

Quartiles of galectin median (range)	Number of cases	Odds ratio (95% CI)[a]
Low: 12.1 (6.4–13.6)	121	1.00
14.6 (13.7–15.6)	123	1.62 (0.92–2.85)
16.7 (15.7–18.3)	115	1.92 (1.08–3.41)
High: 20.9 (18.4–83)	116	2.01 (1.11–3.66)

CI, confidence interval.
[a]Adjusted for age, body mass index, pulse, hypertension, dyslipidaemia, and diabetes.

Discussion

In a prospective cohort study of elderly adults pre-specified to study alterations in cardiovascular structure and function with ageing, our results suggest that Gal-3 may be used to predict odds of myocardial dysfunction associated with myocardial ageing.

In this cross-sectional analysis, we observed impairments in myocardial relaxation,[24] typically seen with ageing when left ventricular filling decreases in early diastole, leading to reductions in the mitral peak early-to-late diastolic filling velocity ratio. In contrast to studies that have reported associations between Gal-3 and cardiovascular conditions within clinical cohorts,[15,16,23,25–28] our studied cohort consisted of community-dwelling elderly adults who did not have clinical heart failure, further supported by relatively low BNP levels. Notably, BNP levels were similar among those with or without impaired myocardial relaxation. Besides, levels of high-sensitivity troponin I assays in the cohort were low and below upper reference limits suggested in other studies and those that rule out coronary artery disease.[29–31]

Age-associated changes in Gal-3 have been reported in other cohorts, and our findings confirm these previous observations. In fact, we provide new findings by demonstrating changes in Gal-3 within age subgroups that correlated with reductions in myocardial function. Our large sample size had allowed depictions of exact quartiles of Gal-3 to be studied in relation to both age and myocardial ageing. This allows future studies to reference their findings in relation to similar reference quartiles. Prior studies among clinical cohorts

Table 2 Univariate and multivariate association with impaired myocardial relaxation

	Univariate		Multivariate	
	Unadjusted OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
Galectin-3	1.09 (1.04–1.14)	<0.0001	1.05 (1.00–1.10)	0.039
Age ≥75 years	2.57 (1.69–3.90)	<0.0001	2.60 (1.64–4.11)	<0.0001
BMI ≥ 23 kg/m^2	1.96 (1.35–2.84)	<0.0001	2.16 (1.44–3.23)	<0.0001
Hypertension	2.17 (1.50–3.13)	<0.0001	1.42 (0.91–2.22)	0.120
Dyslipidaemia	1.86 (1.29–2.68)	0.001	1.39 (0.90–2.16)	0.139
Diabetes mellitus	2.10 (1.32–3.36)	0.002	1.37 (0.79–2.36)	0.263
Heart rate	1.03 (1.01–1.05)	<0.0001	1.04 (1.02–1.06)	<0.0001

BMI, body mass index; CI, confidence interval; OR, odds ratio.

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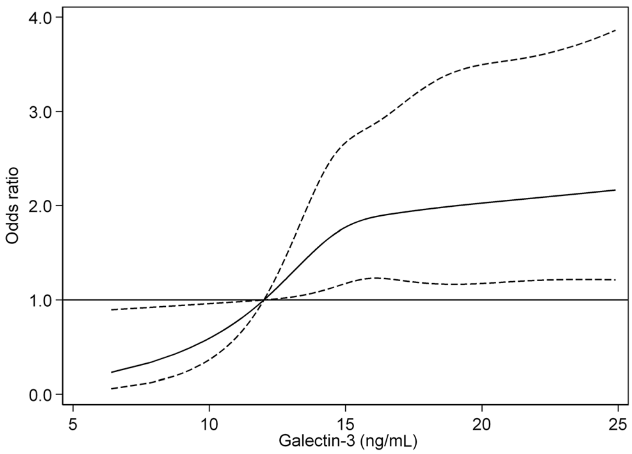
Figure 2 Cubic splines showing association of galectin-3 with impaired myocardial relaxation. The solid dark line represents the odds ratio, and the dotted lines represent 95% confidence intervals. We fitted restricted cubic splines with four knots at the 10th, 36.7th, 63.4th, and 90th percentile in a logistic regression model adjusted for age, body mass index, hypertension, dyslipidaemia, diabetes mellitus, and heart rate.



evaluating the relationship between echocardiographic measures and Gal-3, such as the DEAL-HF trial involving heart failure patients, have found associations between higher levels of Gal-3 and cardiac remodelling.[15] Our data demonstrate a similar graded dose–response relationship between Gal-3 levels and impaired myocardial relaxation. Future studies may use Gal-3 quantitatively to study trajectory of myocardial ageing over time: progressively with chronological age that is upstream prior to disease development and downstream as a marker of progressive myocardial dysfunction.[19]

Galectin-3 is secreted by activated macrophages and modulates several physiological and pathological processes such as inflammation and fibrosis, contributing to the development of cardiovascular conditions such as heart failure.[32,33] By evaluating circulating Gal-3 in tandem with echocardiographic markers of myocardial ageing, we found important links between Gal-3 and specific markers of myocardial function. In the present study, those measures of impaired myocardial relaxation found associated with circulating levels of Gal-3 represent alterations that frequently precede clinical heart failure phenotypes such as heart failure with preserved ejection fraction.[6,34] Data from the Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity Echocardiographic Substudy indicate that diastolic dysfunction as measured by mitral inflow parameters predicted worse outcomes in heart failure with preserved ejection fraction.[35] In relation to Gal-3, Shah *et al.*[36] examined patients with acute dyspnoea and reported associations between Gal-3 and echocardiographic parameters reflective of higher left ventricular filling pressures. Our findings concur with these existing observations. In addition, we provide new data to show that these phenotypic associations between circulating Gal-3 and myocardial alterations exist in preclinical stages. Relatedly, the Cardiovascular Health Study,[37] a community study of older living adults, had demonstrated associations between circulating fibrosis-related biomarkers with future risks of incident cardiovascular disease. Taken together, our data support the notion that detection of preclinical myocardial dysfunction in community-dwelling elderly adults using circulating biomarkers such as Gal-3 may be used as a preventative strategy against incident heart failure with preserved ejection fraction among elderly adults.

Our cross-sectional observations preclude causal and biological inferences. However, impaired relaxation is a common characteristic of the aged heart with increasing recognition that myocardial fibrosis may be a contributing factor.[38] Specifically, interventional studies involving disease models suggest that excess collagen, and not myocyte hypertrophy, contributes to myocardial fibrosis in ageing.[39,40] Gal-3 binds to extracellular proteins[41] and contains collagen-like domains that are substrates for cleavage by matrix metalloproteinases —a group of endopeptidases responsible for matrix protein degradation.[42,43] In rats, intrapericardial infusion of Gal-3 resulted in left ventricular collagen accumulation and reduction in left ventricular ejection fraction.[14] Given that ageing leads to alterations in the cardiac interstitium,[44] higher circulating levels of Gal-3 may reflect increased cell proliferation and collagen production in the myocardium.

Several limitations of this study deserve mention. First, plasma levels of biomarkers were measured at a single time point, and it is possible that the longitudinal trajectory of change in biomarkers may provide additional causal inferences, independent of baseline levels, on future risks of cardiovascular disease in this cohort. Second, we evaluated myocardial function by conventional echocardiography. Imaging modalities such as magnetic resonance imaging[45] may detect myocardial fibrosis with greater sensitivity and specificity, but their use is not easily replicated in large-scale community studies particularly if intravenous administration of imaging contrast is required. Plasma biomarkers such as Gal-3 are readily available and provide a non-invasive assessment of ageing-related fibrosis appropriate for cohort studies.[46] Given current developments in antifibrotic agents,[47,48] clinical trials targeting fibrosis may use plasma Gal-3 to determine effects on fibrosis-related myocardial function, extending upstream as a preventive strategy in preclinical cohorts such as high-risk older adults, identified by Gal-3 levels. Third, we acknowledge that our sample size is limited, consisting of mainly older adults. A control group of younger middle-aged adults, matched for gender, may further strengthen our observations regarding myocardial ageing. Fourth, we had focused on left heart assessment in this analysis only. We did not report information about the right heart.[49] Right heart assessment such as right ventricular strain and right ventricular systolic pressure has been previously associated with Gal-3.[50] Importantly, Gal-3 has also been associated with preclinical metabolic heart disease among young obese patients who had abnormalities in right ventricular coupling.[51] Thus, right heart assessment may have added important insights into right heart ageing as a preclinical manifestation of ageing-related heart dysfunction. Fifth, our study sample consisted of older adults of ethnic Asian Chinese descent, for which results may not be applicable to participants of different ethnic origins within Asia. Given that Gal-3 may be associated with cardiovascular outcomes differentially by race,[52] future studies incorporating different Asian ethnic groups may be necessary to qualify our observations better among Asians. Finally, while we observed no association between microalbuminuria and myocardial dysfunction in this cohort, we acknowledge that renal clearance of plasma Gal-3 may be impaired in certain populations[25,53] and more specific measures of kidney function could have been useful for further multivariable adjustments.

However, our study has several strengths. We conducted our study in a large well-characterized community-based population. The large sample size contributed to statistical power and adjustments for confounders. The collection of biomarkers occurred simultaneously with clinical assessment, reducing likelihood of misclassification bias.

In conclusion, Gal-3, in the setting of elderly adults, is associated with impairments in myocardial function related to ageing. Our findings provide support for future research into pathways represented by circulating biomarkers such as Gal-3 to detect cardiovascular disease upstream in order to reduce cardiovascular disease downstream in ageing populations. In the setting of clinical trials, Gal-3 could potentially be used to identify target populations for early interventions.

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Conflict of interest

None declared.

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Supporting information

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

Table S1. Associations between galectin-3 and echocardiographic parameters.

References

1. Lakatta EG. Changes in cardiovascular function with aging. *Eur Heart J* 1990; **11**: 22–29.
2. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: part I: aging arteries: a “set up” for vascular disease. *Circulation* 2003; **107**: 139–146.
3. Lakatta EG, Wang M, Najjar SS. Arterial aging and subclinical arterial disease are fundamentally intertwined at macroscopic and molecular levels. *Med Clin North Am* 2009; **93**: 583–604.
4. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: part II: the aging heart in health: links to heart disease. *Circulation* 2003; **107**: 346–354.
5. Arbab-Zadeh A, Dijk E, Prasad A, Fu Q, Torres P, Zhang R, Thomas JD, Palmer D, Levine BD. Effect of aging and physical activity on left ventricular compliance. *Circulation* 2004; **110**: 1799–1805.
6. Kitzman DW, Gardin JM, Gottdiener JS, Arnold A, Boineau R, Aurigemma G, Marino EK, Lyles M, Cushman M, Enright PL. Importance of heart failure with preserved systolic function in patients ≥65 years of age. CHS Research Group. Cardiovascular Health Study. *Am J Cardiol* 2001; **87**: 413–419.
7. Redfield MM, Jacobsen SJ, Burnett JC Jr, Mahoney DW, Bailey KR, Rodeheffer RJ. Burden of systolic and diastolic ventricular dysfunction in the community: appreciating the scope of the heart failure epidemic. *JAMA* 2003; **289**: 194–202.
8. Cherubini A, Oristrell J, Pla X, Ruggiero C, Ferretti R, Diestre G, Clarfield AM, Crome P, Hertogh C,

DOI: 10.1002/ehf2.12495

Lesauskaite V, Prada GI, Szczerbinska K, Topinkova E, Sinclair-Cohen J, Edbrooke D, Mills GH. The persistent exclusion of older patients from ongoing clinical trials regarding heart failure. *Arch Intern Med* 2011; **171**: 550–556.

9. Herrera AP, Snipes SA, King DW, Torres-Vigil I, Goldberg DS, Weinberg AD. Disparate inclusion of older adults in clinical trials: priorities and opportunities for policy and practice change. *Am J Public Health* 2010; **100**: S105–S112.
10. Konrat C, Boutron I, Trinquart L, Auleley GR, Ricordeau P, Ravaud P. Underrepresentation of elderly people in randomised controlled trials. The example of trials of 4 widely prescribed drugs. *PLoS One* 2012; **7**: e33559.
11. Kolatsi-Joannou M, Price KL, Winyard PJ, Long DA. Modified citrus pectin reduces galectin-3 expression and disease severity in experimental acute kidney injury. *PLoS One* 2011; **6**: e18683.
12. Kusaka H, Yamamoto E, Hirata Y, Fujisue K, Tokitsu T, Sugamura K, Sakamoto K, Tsujita K, Kaikita K, Hokimoto S, Sugiyama S, Ogawa H. Clinical significance of plasma galectin-3 in patients with coronary artery disease. *Int J Cardiol* 2015; **201**: 532–534.
13. Liu YH, D'Ambrosio M, Liao TD, Peng H, Rhaleb NE, Sharma U, Andre S, Gabius HJ, Carretero OA. *N*-acetyl-seryl-aspartyl-lysyl-proline prevents cardiac remodeling and dysfunction induced by galectin-3, a mammalian adhesion/growth-regulatory lectin. *Am J Physiol Heart Circ Physiol* 2009; **296**: H404–H412.
14. Sharma UC, Pokharel S, van Brakel TJ, van Berlo JH, Cleutjens JP, Schroen B, Andre S, Crijns HJ, Gabius HJ, Maessen J, Pinto YM. Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction. *Circulation* 2004; **110**: 3121–3128.
15. Lok DJ, Van Der Meer P, de la Porte PW, Lipsic E, Van Wijngaarden J, Hillege HL, van Veldhuisen DJ. Prognostic value of galectin-3, a novel marker of fibrosis, in patients with chronic heart failure: data from the DEAL-HF study. *Clin Res Cardiol* 2010; **99**: 323–328.
16. Ueland T, Aukrust P, Broch K, Aakhus S, Skardal R, Muntendam P, Gullestad L. Galectin-3 in heart failure: high levels are associated with all-cause mortality. *Int J Cardiol* 2011; **150**: 361–364.
17. Yu L, Ruifrok WP, Meissner M, Bos EM, van Goor H, Sanjabi B, van der Harst P, Pitt B, Goldstein IJ, Koerts JA, van Veldhuisen DJ, Bank RA, van Gilst WH, Sillje HH, de Boer RA. Genetic and pharmacological inhibition of galectin-3 prevents cardiac remodeling by interfering with myocardial fibrogenesis. *Circ Heart Fail* 2013; **6**: 107–117.
18. de Boer RA, van Veldhuisen DJ, Gansevoort RT, Muller Kobold AC, van Gilst WH, Hillege HL, Bakker SJ, van der Harst P. The fibrosis marker galectin-3 and outcome in the general population. *J Intern Med* 2012; **272**: 55–64.
19. Sanchis-Gomar F, Santos-Lozano A, Pareja-Galeano H, Garatachea N, Alis R, Fiuza-Luces C, Moran M, Emanuele E, Lucia A. Galectin-3, osteopontin and successful aging. *Clin Chem Lab Med* 2016 May; **54**: 873–877.
20. Koh AS, Gao F, Liu J, Fridianto KT, Ching J, Tan RS, Wong JI, Chua SJ, Leng S, Zhong L, Keng BM, Huang FQ, Yuan JM, Koh WP, Kovalik JP. Metabolomic profile of arterial stiffness in aged adults. *Diab Vasc Dis Res* 2018; **15**: 74–80.
21. Koh AS, Gao F, Leng S, Kovalik JP, Zhao X, Tan RS, Fridianto KT, Ching J, Chua SJ, Yuan JM, Koh WP, Zhong L. Dissecting clinical and metabolomics associations of left atrial phasic function by cardiac magnetic resonance feature tracking. *Sci Rep* 2018; **8**: 8138–26456.
22. Hankin JH, Stram DO, Arakawa K, Park S, Low SH, Lee HP, Yu MC. Singapore Chinese Health Study: development, validation, and calibration of the quantitative food frequency questionnaire. *Nutr Cancer* 2001; **39**: 187–195.
23. Djousse L, Matsumoto C, Petrone A, Weir NL, Tsai MY, Gaziano JM. Plasma galectin 3 and heart failure risk in the Physicians' Health Study. *Eur J Heart Fail* 2014; **16**: 350–354.
24. Nagueh SF, Smiseth OA, Appleton CP, Byrd BF III, Dokainish H, Edvardsen T, Flachskampf FA, Gillebert TC, Klein AL, Lancellotti P, Marino P, Oh JK, Popescu BA, Waggoner AD. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr* 2016; **29**: 277–314.
25. Gopal DM, Kommineni M, Ayalon N, Koelbl C, Ayalon R, Biolo A, Dember LM, Downing J, Siwik DA, Liang CS, Colucci WS. Relationship of plasma galectin-3 to renal function in patients with heart failure: effects of clinical status, pathophysiology of heart failure, and presence or absence of heart failure. *J Am Heart Assoc* 2012; **1**: e000760.
26. de Boer RA, Lok DJ, Jaarsma T, Van Der Meer P, Voors AA, Hillege HL, van Veldhuisen DJ. Predictive value of plasma galectin-3 levels in heart failure with reduced and preserved ejection fraction. *Ann Med* 2011; **43**: 60–68.
27. Lopez-Andres N, Rossignol P, Iraqi W, Fay R, Nuee J, Ghio S, Cleland JG, Zannad F, Lacolley P. Association of galectin-3 and fibrosis markers with long-term cardiovascular outcomes in patients with heart failure, left ventricular dysfunction, and dyssynchrony: insights from the CARE-HF (Cardiac Resynchronization in Heart Failure) trial. *Eur J Heart Fail* 2012; **14**: 74–81.
28. McCullough PA, Olobatoke A, Vanhecke TE. Galectin-3: a novel blood test for the evaluation and management of patients with heart failure. *Rev Cardiovasc Med* 2011; **12**: 200–210.
29. Aw TC, Phua SK, Tan SP. Measurement of cardiac troponin I in serum with a new high-sensitivity assay in a large multi-ethnic Asian cohort and the impact of gender. *Clin Chim Acta* 2013; **422**: 26–28.
30. de Lemos JA, deFilippi CR. Prevalence and significance of detectable troponins as measured by highly sensitive assays in the general population. *Coron Artery Dis* 2013; **24**: 705–709.
31. Hochholzer W, Valina CM, Stratz C, Amann M, Schlittenhardt D, Buttner HJ, Trenk D, Neumann FJ. High-sensitivity cardiac troponin for risk prediction in patients with and without coronary heart disease. *Int J Cardiol* 2014; **176**: 444–449.
32. Wu CK, Su MY, Lee JK, Chiang FT, Hwang JJ, Lin JL, Chen JJ, Liu FT, Tsai CT. Galectin-3 level and the severity of cardiac diastolic dysfunction using cellular and animal models and clinical indices. *Sci Rep* 2015; **5**: 17007.
33. Yang RY, Rabinovich GA, Liu FT. Galectins: structure, function and therapeutic potential. *Expert Rev Mol Med* 2008; **10**: e17.
34. Zile MR, Baicu CF, Gaasch WH. Diastolic heart failure—abnormalities in active relaxation and passive stiffness of the left ventricle. *N Engl J Med* 2004; **350**: 1953–1959.
35. Persson H, Lonn E, Edner M, Baruch L, Lang CC, Morton JJ, Ostergren J, McKelvie RS. Diastolic dysfunction in heart failure with preserved systolic function: need for objective evidence: results from the CHARM Echocardiographic Substudy—CHARMES. *J Am Coll Cardiol* 2007; **49**: 687–694.
36. Shah RV, Chen-Tournoux AA, Picard MH, van Kimmenade RR, Januzzi JL. Galectin-3, cardiac structure and function, and long-term mortality in patients with acutely decompensated heart failure. *Eur J Heart Fail* 2010; **12**: 826–832.
37. Agarwal I, Glazer NL, Barasch E, Biggs ML, Djousse L, Fitzpatrick AL, Gottdiener JS, Ix JH, Kizer JR, Rimm EB, Sicovick DS, Tracy RP, Mukamal KJ. Fibrosis-related biomarkers and incident cardiovascular disease in older adults: the cardiovascular health study. *Circ Arrhythm Electrophysiol* 2014; **7**: 583–589.
38. Nicoletti A, Michel JB. Cardiac fibrosis and inflammation: interaction with hemodynamic and hormonal factors. *Cardiovasc Res* 1999; **41**: 532–543.
39. Martos R, Baugh J, Ledwidge M, O'Loughlin C, Conlon C, Patle A, Donnelly SC, McDonald K. Diastolic heart failure: evidence of increased myocardial collagen turnover linked to diastolic dysfunction. *Circulation* 2007; **115**: 888–895.

40. Yamamoto K, Masuyama T, Sakata Y, Nishikawa N, Mano T, Yoshida J, Miwa T, Sugawara M, Yamaguchi Y, Ookawara T, Suzuki K, Hori M. Myocardial stiffness is determined by ventricular fibrosis, but not by compensatory or excessive hypertrophy in hypertensive heart. *Cardiovasc Res* 2002 July; **55**: 76–82.
41. Ochieng J, Furtak V, Lukyanov P. Extracellular functions of galectin-3. *Glycoconj J* 2002; **19**: 527–535.
42. Puthenedam M, Wu F, Shetye A, Michaels A, Rhee KJ, Kwon JH. Matrilysin-1 (MMP7) cleaves galectin-3 and inhibits wound healing in intestinal epithelial cells. *Inflamm Bowel Dis* 2011; **17**: 260–267.
43. Ochieng J, Fridman R, Nangia-Makker P, Kleiner DE, Liotta LA, Stetler-Stevenson WG, Raz A. Galectin-3 is a novel substrate for human matrix metalloproteinases-2 and -9. *Biochemistry* 1994; **33**: 14109–14114.
44. Macri SC, Bailey CC, de Oca NM, Silva NA, Rosene DL, Mansfield KG, Miller AD. Immunophenotypic alterations in resident immune cells and myocardial fibrosis in the aging rhesus macaque (*Macaca mulatta*) heart. *Toxicol Pathol* 2012; **40**: 637–646.
45. Liu CY, Liu YC, Wu C, Armstrong A, Volpe GJ, van der Geest RJ, Liu Y, Hundley WG, Gomes AS, Liu S, Nacif M, Bluemke DA, Lima JAC. Evaluation of age-related interstitial myocardial fibrosis with cardiac magnetic resonance contrast-enhanced T1 mapping: MESA (Multi-Ethnic Study of Atherosclerosis). *J Am Coll Cardiol* 2013; **62**: 1280–1287.
46. Ho JE, Liu C, Lyass A, Courchesne P, Pencina MJ, Vasan RS, Larson MG, Levy D. Galectin-3, a marker of cardiac fibrosis, predicts incident heart failure in the community. *J Am Coll Cardiol* 2012; **60**: 1249–1256.
47. Richeldi L, du Bois RM. Pirfenidone in idiopathic pulmonary fibrosis: the CAPACITY program. *Expert Rev Respir Med* 2011; **5**: 473–481.
48. Sharma K, Ix JH, Mathew AV, Cho M, Pflueger A, Dunn SR, Francos B, Sharma S, Falkner B, McGowan TA, Donohue M, Ramachandrarao S, Xu R, Fervenza FC, Kopp JB. Pirfenidone for diabetic nephropathy. *J Am Soc Nephrol* 2011; **22**: 1144–1151.
49. Gorter TM, van Veldhuisen DJ, Bauersachs J, Borlaug BA, Celutkiene J, Coats AJS, Crespo-Leiro MG, Guazzi M, Harjola VP, Heymans S, Hill L, Lainscak M, Lam CSP, Lund LH, Lyon AR, Mebazaa A, Mueller C, Paulus WJ, Pieske B, Piepoli MF, Ruschitzka F, Rutten FH, Seferovic PM, Solomon SD, Shah SJ, Triposkiadis F, Wachter R, Tschöpe C, de Boer RA. Right heart dysfunction and failure in heart failure with preserved ejection fraction: mechanisms and management. Position statement on behalf of the Heart Failure Association of the European Society of Cardiology. *Eur J Heart Fail* 2018; **20**: 16–37.
50. Fenster BE, Lasalvia L, Schroeder JD, Smyser J, Silveira LJ, Buckner JK, Brown KK. Galectin-3 levels are associated with right ventricular functional and morphologic changes in pulmonary arterial hypertension. *Heart Vessels* 2016; **31**: 939–946.
51. Gopal DM, Ayalon N, Wang YC, Siwik D, Sverdlov A, Donohue C, Perez A, Downing J, Apovian C, Silva V, Panagia M, Kolachalama V, Ho JE, Liang CS, Gokce N, Colucci WS. Galectin-3 is associated with stage B metabolic heart disease and pulmonary hypertension in young obese patients. *J Am Heart Assoc* 2019; **8**: e011100.
52. McEvoy JW, Chen Y, Halushka MK, Christenson E, Ballantyne CM, Blumenthal RS, Christenson RH, Selvin E. Galectin-3 and risk of heart failure and death in blacks and whites. *J Am Heart Assoc* 2016; **5**: e003079.
53. Meijers WC, van der Velde AR, Ruifrok WP, Schroten NF, Dokter MM, Damman K, Assa S, Franssen CF, Gansevoort RT, van Gilst WH, Sillje HH, de Boer RA. Renal handling of galectin-3 in the general population, chronic heart failure, and hemodialysis. *J Am Heart Assoc* 2014; **3**: e000962.

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