Supplement to Usefulness of Released Cardiac Myosin Binding Protein-C as a Predictor of Cardiovascular Events

Carl W. Tong MD PhD[a,b*], Giuseppina F. Dusio PhD[c#], Suresh Govindan PhD[d#], Dustin W. Johnson MD[b], David T. Kidwell MD[b], Lisa M. De La Rosa MD[b], Paola C. Rosas MD PhD RPh[a], Yang Liu MD PhD[a], Elizabeth Ebert MD[b], M. Karen Newell-Rogers MD[a], Jeffrey B. Michel MD[b], Jerome P. Trzeciakowski PhD[a], Sakthivel Sadayappan PhD[d,e*]

[a] Department of Medical Physiology, Texas A&M University Health Science Center College of Medicine, Temple, TX; [b] Baylor Scott & White Health – Central Texas, Internal Medicine/Cardiology Division, Temple, TX; [c] University of Arkansas for Medical Sciences, Little Rock, AR; [d]Department of Cell and Molecular Physiology, Health Sciences Division, Loyola University Chicago, Maywood, IL; [e]Department of Internal Medicine, Heart, Lung and Vascular Institute, Cardiovascular Center, University of Cincinnati College of Medicine, Cincinnati, OH

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*# Equally contributed

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Address for correspondence: Sakthivel Sadayappan, PhD, MBA. Department of Internal Medicine, Heart, Lung and Vascular Institute, Cardiovascular Center, University of Cincinnati College of Medicine, 231 Albert Sabin Way, Cincinnati, OH 45267-0575, USA; Phone: +1 513-558-7498; FAX: +1 513-558-2884; Email: sadayasl@ucmail.uc.edu

Table S1: Diagnoses Encountered for Ordering Exercise Stress Echocardiography

ICD-10	No	%	Diagnosis Description
E66.01	1	0.63%	Drug induced obesity
I10	2	1.27%	Essential Hypertension
I13.1	1	0.63%	Hypertensive heart and chronic kidney disease without heart failure, stage 1 through 4
I20.8	7	4.43%	Other forms of angina pectoris
I25.10	31	19.62%	Atherosclerotic heart disease of native coronary artery without angina pectoris
I25.119	1	0.63%	Atherosclerotic heart disease of native coronary artery with unspecified angina pectoris
I25.810	1	0.63%	Atherosclerotic heart disease of bypass graft without angina pectoris
I34.0	1	0.63%	Non rheumatic mitral valve insufficiency
I35.0	1	0.63%	Non rheumatic aortic valve stenosis
I48.0	2	1.27%	Paroxysmal atrial fibrillation
I48.91	2	1.27%	Unspecified atrial fibrillation
I49.5	1	0.63%	Sick Sinus Syndrome
I49.8	1	0.63%	Other specified cardiac arrhythmias
I49.9	1	0.63%	Cardiac arrhythmia, unspecified
R00.2	2	1.27%	Palpitations
R06.00	3	1.90%	Dyspnea, unspecified
R06.02	5	3.16%	Shortness of Breath
R06.09	4	2.53%	Other forms of Dyspnea
R06.2	1	0.63%	Wheezing
R06.9	1	0.63%	Unspecified abnormalities of breathing
R07.82	1	0.63%	Intercostal pain
R07.89	3	1.90%	Other chest pain
R07.9	78	49.37%	Chest Pain, unspecified
R42	1	0.63%	Dizziness and giddiness
R53.81	1	0.63%	Other malaise
R55	1	0.63%	Syncope and Collapse
R94.31	1	0.63%	Abnormal ECG
Z01.810	2	1.27%	Encounter for pre-procedural cardiovascular examination
Z01.818	1	0.63%	Encounter for other pre-procedure examination

Due to concerns for patients' safety, the study institution's protocol excluded patients with known low left ventricular ejection fraction (LVEF) from exercise stress study. Thus, narrow range of normal LVEFs will not provide bases for good correlation analysis. However, Sa (tissue Doppler of maximum myocardial contractile velocity about the mitral valve annulus during systole), which corresponds to +dP/dtmax of contraction,[1] can be used as measure of contractility. The basal tissue Doppler Sa correlated with pre-stress cMyBP-C (Table S2). This correlation grew stronger when subjects who suffered the primary outcome were removed from the analysis (Table S2). The basal tissue Doppler e', which is peak myocardial relaxation velocity during early diastole, did not correlate with pre-stress cMyBP-C. The basal E/e' ratio, where E is the peak blood flow velocity Doppler during early diastole, did not correlate with pre-stress cMyBP-C. Thus, contractility positively correlated with pre-stress cMyBP-C levels but diastolic function (e' and E/e')[2] did not correlate with pre-stress cMyBP-C levels.

Table S2: Correlation of Basal Echocardiography to Pre-Stress Serum Cardiac Myosin Binding Protein-C Levels

Variables	**Pearson Correlation r**	**Two-tail p**
Pre-Stress Sa *vs.* Pre-Stress cMyBP-C	0.161	0.047*
Pre-Stress Sa *vs.* Pre-Stress cMyBP-C in subjects who did not suffer a primary outcome event	0.276	0.002*
Pre-Stress e' *vs.* Pre-Stress cMyBP-C	0.026	0.753
Pre-Stress e' *vs.* Pre-Stress cMyBP-C in subjects who did not suffer a primary outcome event	0.115	0.197
Pre-Stress E/e' *vs.* Pre-Stress cMyBP-C	-0.087	0.289
Pre-Stress E/e' *vs.* Pre-Stress cMyBP-C in subjects who did not suffer a primary outcome event	-0.169	0.057

Cardiac Myosin Binding Protein-C (cMyBP-C). e': tissue Doppler of peak myocardial relaxation velocity about the mitral valve annulus during early diastole. E: Doppler of peak blood inflow velocity to the left ventricle across the mitral valve during early diastole. Sa: tissue Doppler of peak myocardial contraction velocity during systole. * $p<0.05$ for existence of significant correlation.

There are 9 subjects in the study who did not have cardiovascular risk factors, exhibited normal echocardiographic findings, performed normal exercise stress echocardiography, and did not suffer primary outcome. Consequently, these 9 subjects can be used as true normal for the study. This true normal population appeared to differ from the rest of the study population by lower cMyBP-C levels. Like rest of the study population, exercise stress also increased serum cMyBP-C in normal subgroup. However, the apparent lower cMyBP-C levels are significant only by using unequal variances based analysis (See Table S3).

Table S3: Comparison of True Normal to Rest of Study Population

	True Normal n=9	**All Others n=149**	**P for unequal variances**	**P for equal variances**
Pre-Stress cMyBP-C (ng/ml)	205±40	708±113*	<0.001	0.278
Post-Stress cMyBP-C (ng/ml)	254±41#	997±155*#	<0.001	0.241
Absolute Change in cMyBP-C (ng/ml)	49±6	289±50*	<0.001	0.270
% Change in cMyBP-C	29±5	53±7	0.007	0.410

*denotes significant difference $p<0.05$ from true normal (chose p for unequal variance if standard deviations were very different). # denotes significant difference $p<0.05$ from its corresponding pre-stress values. Values are Mean ± SE.

Power analyses were done to assess the ability of serum cMyBP-C in differentiating a population that is more likely to suffer cardiovascular disease event from a normal population. Versions of SPSS and Stata used for the analyses did not have the capability to perform power analyses on this study's particular type of data. Thus, power analyses were done manually.

For estimating the sample size needed for a PreC or PostC as dichotomous covariates, we used the formula developed by Schoenfeld (1983)[3]:

$$n = \frac{\left(z_{1-\alpha/2} + z_{1-\beta}\right)^2}{PR(1-R)(\log B)^2}$$

Where, as above, α = significance level (.05); (1 − β) = desired power; B = Hazard ratio to be detected (8.1 for PreC; 4.8 for PostC); P = proportion of subjects exhibiting the primary outcome; R = proportion of subjects for whom PreC = 1 (0.77) or PostC = 1 (0.57) - i.e. number of subjects with [cMyBP-C] above the respective threshold concentrations. For multiple Cox Regresssion, the estimated *n*'s were adjusted based on the Pseudo-R^2 between PreC or PostC and the remaining covariates. We then used the known n=158, R, α = 0.05, and hazard ratio to calculate power = (1 − β). The calculated power is > 0.999 for both PreC and PostC.

Because cMyBP-C follows a log-normal distribution, we used log10([cMyBP-C]) in our Cox regression and power analysis. We use the following formula (Hsieh and Lavori (2000))[4] for the estimated sample size *n* for a continuous covariate *x* when *x* is normally distributed:

$$n = \frac{\left(z_{1-\alpha/2} + z_{1-\beta}\right)^2}{P\sigma^2(\log B)^2}$$

Where α = significance level (.05); $(1-\beta)$ = desired power; B = Hazard ratio to be detected (13.8 for pre-stress or 14.3 for post-stress); P = proportion of subjects exhibiting critical events; σ = variance of x (0.719 for pre-stress log10[cMyBP-C] or 0.727 for post-stress log10([cMyBP-C]). For multiple Cox Regresssion, the estimated *n*'s were adjusted based on the multiple correlation coefficient, ρ, between log[cMyBP-C] and the remaining covariates:

$$n^* = \frac{n}{1-\rho^2}$$

We then used the known n=158, R, $\alpha = 0.05$, and hazard ratios to calculate power = $(1-\beta)$. The calculated power is >0.999 for both pre-stress and post-stress cMyBP-C levels.

Although the power analyses suggest that circulating cMyBP-C has >99% chance of finding population differences, we caution about exuberance of its potential use. The positive findings of the current study population of patients who were referred for stress echocardiography likely caused the very high calculated power. A more generalize population may provide a different yield. Power provides a measure on the ability of a method to differentiate between populations; however, the ability of serum cMyBP-C to predict outcome on individual bases will vary.

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