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Increasing Serum Soluble Angiotensin Converting Enzyme 2 Activity following Intensive Medical Therapy is Associated with Better Prognosis in Acute Decompensated Heart Failure

Zhili Shao, MD, PhD¹, Kevin Shrestha, MD², Allen G. Borowski, RDCS², David P. Kennedy, PhD¹, Slava Epelman, MD, PhD³, James D. Thomas, MD², and W. H. Wilson Tang, MD^{1,2}

¹Center for Cardiovascular Diagnostics and Prevention, Department of Cell Biology, Lerner Research Institute, Cleveland Clinic, Cleveland Ohio

²Department of Cardiovascular Medicine, Heart and Vascular Institute, Cleveland Clinic, Cleveland Ohio

³Cardiovascular Division Washington University School of Medicine, St. Louis Missouri

Abstract

Background—Angiotensin-converting enzyme 2 (ACE2) is an endogenous counter-regulator of the renin-angiotensin system that has been recently identified in circulating form. We aim to investigate the relationship between changes in soluble ACE2 (sACE2) activity, myocardial performance and long-term clinical outcomes in patients with acute decompensated heart failure (ADHF). We hypothesize that increasing sACE2 activity levels during intensive medical treatment is associated with improved myocardial performance and long-term clinical outcomes.

Methods and Results—In 70 patients admitted to the intensive care unit with ADHF, serum sACE2 activity levels, echocardiographic data, and hemodynamic variables were collected within 12 hours of admission (n=70) and 48–72 hours following enrollment after intensive medical treatment (n=57). The median baseline and 48–72 hour serum sACE2 activity levels were 32 [23, 43] ng/mL and 40 [28, 60] ng/mL, respectively. Baseline serum sACE2 activity levels correlated with surrogate measures of right ventricular (RV) diastolic dysfunction, including right atrial volume index (RAVi) (r=0.31, p=0.010), tricuspid E/A ratio (r=0.39, p=0.007), as well as B-type natriuretic peptide (r=0.32, p=0.008). However, there were no correlations between serum sACE2 and left ventricular (LV) systolic or diastolic dysfunction. Following intensive medical therapy, a 50% increase in baseline serum sACE2 levels predicted a significant reduction in risk of death, cardiac transplantation, or ADHF re-hospitalization, including after adjustment for baseline age, RAVi, and BNP levels (Hazard ratio 0.35, 95% confidence interval 0.12–0.84, p=0.018).

Conclusion—In patients admitted with ADHF, increasing serum sACE2 activity levels during intensive medical therapy predict improved outcomes independent of underlying cardiac indices.

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Address for correspondence: W. H. Wilson Tang, MD, 9500 Euclid Avenue, Desk J3-4, Cleveland, OH 44195. Phone: (216) 444-2121, Fax: (216) 445-6165, tangw@ccf.org.

CONFLICT OF INTEREST STATEMENT

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Keywords

Soluble angiotensin converting enzyme 2; acute decompensated heart failure; right ventricular diastolic dysfunction

INTRODUCTION

Angiotensin converting enzyme 2 (ACE2) was discovered from human heart failure (HF) ventricle cDNA library as the only human homologue of ACE[1]. Like angiotensin converting enzyme (ACE), ACE2 is a carboxypeptidase and type I transmembrane protein with an extracellular N-terminal domain containing the active site[2]. Instead of converting angiotensin I into angiotensin II (Ang II), ACE2 showed the highest catalytic efficacy in conversion of Ang II to angiotensin 1–7 (Ang 1–7)[3]. Ang 1–7 binds to Mas receptor, thereby exerting vasodilatory, cardioprotective effects opposite to Ang II[4]. ACE2 can be secreted into circulation as an enzymatically active ectodomain ACE2 (sACE2)[2]. Circulating sACE2 activity is readily detectable in humans, and our group and others have shown its heightened activity levels in the setting of chronic systolic HF[5–6]. More importantly, circulating sACE2 activity levels have been shown to be an independent predictor of adverse clinical events in patients with chronic HF[7]. However, the relationship between sACE2 activity (particularly with its changes following treatment), cardiac performance, and long-term clinical outcomes remains unclear. In patients admitted with acute decompensated heart failure, we aimed to investigate the relationship between admission sACE2 activity and its change after intensive medical therapy with myocardial performance and long-term clinical outcomes in patients. We hypothesized that increasing sACE2 activity levels during intensive medical treatment predict improved myocardial performance and long-term clinical outcomes.

METHODS

Study population

Seventy consecutive adult patients (≥ 18 years of age) with ADHF admitted within 12 hours of admission to the Cleveland Clinic HF intensive care unit (ICU) were prospectively recruited for this study. All provided written informed consent approved by the Cleveland Clinic Institutional Review Board. Inclusion criteria included markedly impaired left ventricular systolic function defined by left ventricular ejection fraction (LVEF) < 30%. We excluded patients on artificial ventilation, those who had undergone aortic and/or mitral valve repair or prosthesis or cardiac transplantation.

Hemodynamic Measurements

A 7Fr fluid-filled pulmonary artery catheter was placed for clinical indications as part of the hemodynamic guided therapy at the HF intensive care unit. Hemodynamic data were collected at baseline and up to 48–72 hours of follow-up, including systemic blood pressure, central venous pressure, systolic and mean pulmonary artery pressures (PAP), and pulmonary capillary wedge pressure (PCWP, represented the average of 5 cycles with balanced transducers). Cardiac index was calculated with the Fick equation through sampling of a mixed central venous blood gas taken in the pulmonary artery while assuming standard metabolic rates.

Transthoracic Echocardiography

A comprehensive two-dimensional echocardiography was performed with a commercially available system (Vingmed, System Seven, General Electric, Piscataway, NJ) by a single

American Society of Echocardiography registered research sonographer (A.B.) at the time of hemodynamic measurement. Images were acquired in the left lateral decubitus position with a phased-array transducer in the standard parasternal and apical views. Standard two-dimensional and Doppler data, triggered to the QRS complex, were digitally stored in a cine-loop format.

Sample collection and serum sACE2 activity assay

Fasting blood samples were collected at the time of hemodynamic and echocardiographic evaluation (within 12 hours of admission and at 48–72 hours thereafter) and allowed to sit at room temperature for at least 30 minutes to clot. After centrifuge, serum was isolated and stored at -80°C and thawed on ice before assay. The ACE2-specific quenched fluorescent substrate protocol was performed as previously described with some modification[5]. Donor human serum (Innovative Research, Novi MI) and patients' serum samples were diluted in a ratio of 3:7 (sample:buffer) with enzyme buffer (1 mol/L NaCl, 75 mmol/L Tris-HCl, 0.5 mmol/L ZnCl_2 , pH 6.5) in the presence of protease inhibitors, including captopril 10 $\mu\text{mol/L}$, amastatin 5 $\mu\text{mol/L}$, bestatin 10 $\mu\text{mol/L}$ (all from Sigma, St. Louis, Missouri), and Z-prolyl-prolinal 10 $\mu\text{mol/L}$ (Biomol International, Plymouth Meeting PA). Each standard and sample has two duplicates with or without the human ACE2-specific inhibitor DX600 (Phoenix Pharmaceuticals, Burlingame CA) with a final concentration of 1 $\mu\text{mol/L}$. Samples were incubated with the quenched fluorogenic peptide substrate (R&D Systems, Minneapolis MN) diluted in enzyme buffer (final concentration 50 $\mu\text{mol/L}$ in 100 μl) at room temperature. Maximal fluorescence (SpectraMax GeminiXS, Molecular Devices, Wokingham, United Kingdom) was determined by experimentation ($\lambda_{\text{ex}} = 324 \text{ nm}$, $\lambda_{\text{em}} = 430 \text{ nm}$, using a 420nm cutoff filter). Serum sACE2 activity was determined at 24 h with 0 h (first test) as the baseline. Values were normalized to a recombinant ACE2 (R&D Systems, Minneapolis MN) standard curve. Intra-assay variability was $5.1 \pm 2.4\%$, and interassay variability was $8.0 \pm 5.5\%$.

Statistical analyses

Continuous variables were summarized as mean $\pm$ standard deviation if normally distributed, and as median and interquartile range [25th, 75th percentile] if non-normally distributed. Normality was assessed by the Shapiro-Wilk W test. Differences continuous variables across clinical categories were assessed using the Wilcoxon rank-sum test. Univariate Spearman's correlation analysis was used to determine the correlation between sACE2 activity and echocardiographic and hemodynamic variables. Adverse clinical events including all-cause mortality, cardiac transplantation or first HF re-hospitalization were prospectively tracked for 1 year by outpatient clinic follow-up, telephone follow-up or chart review. Cox proportional hazard analysis was used to assess risk of adverse clinical events associated with changes in sACE2 activity. Kaplan-Meier survival plots were calculated from baseline to time of any adverse clinical event. All p values reported are from two-sided tests and a p-value <0.05 was considered statistically significant. The p-values in this manuscript are not corrected for multiple hypothesis testing. Statistical analyses were performed using JMP Pro 10.0.0 (SAS Institute, Cary, NC).

RESULTS

Study population

Table 1 illustrates the baseline clinical characteristics of our study population. Upon admission for ADHF, the majority of patients had NYHA function class III (42%) and IV (53%) presentation. More than half (53%) had ischemic etiology. Median baseline and 48–72 hour serum sACE2 activity levels were 32 [23, 43] and 40 [28, 60] ng/mL respectively. In matched pairs analysis, this increase in sACE2 activity was significant ($sACE2 = 6$ [2,

17] ng/mL, $p=0.002$). Baseline sACE2 activity levels were lower in patients with past medical history of hypertension (24 [18, 40] versus 34 [27, 57] mg/mL, $p=0.026$). Admission serum sACE2 activity or changes in serum sACE2 activity after intensive medical treatment were not associated with age, gender, ethnicity, BMI, eGFR, past medical history of diabetes mellitus, NYHA class, or baseline medication use ($p>0.10$ for all). Baseline or changes in sACE2 activity levels after intensive medical treatment were not associated with any sole inpatient medication use including ACEi and/or ARB, aspirin or statin, beta-blocker, loop or thiazide diuretic, aldosterone antagonist, inotropes or vasodilators ($p>0.10$ for all).

Correlation of baseline serum sACE2 activity with hemodynamic, echocardiographic, and plasma BNP levels

Table 2 illustrates the correlation between baseline sACE2 activity and baseline hemodynamic, echocardiographic, and plasma BNP levels. We observed that serum sACE2 activity levels were modestly correlated with BNP levels at admission ($r=0.32$, $p=0.008$, Figure 1) and after 48–72 hours ($r=0.28$, $p=0.040$). However, sACE2 activity levels were not associated with left ventricular (LV) systolic or diastolic dysfunction. Furthermore, serum sACE2 activity correlated with indices of right ventricular (RV) diastolic dysfunction, including right atrial volume index (RAVi, Spearman's $r=0.31$, $p=0.010$) and tricuspid E/A ratio ($r=0.39$, $p=0.007$, Figure 1). Serum sACE2 activity also modestly correlated with RV end-diastolic area ($r=0.26$, $p=0.045$) and systemic vascular resistance ($r=0.29$, $p=0.017$). There were no other correlations were observed with other echocardiographic or hemodynamic parameters at baseline. In multivariable linear regression analysis, baseline sACE2 activity was associated only with baseline BNP levels (standardized $\beta=0.40$, $p=0.002$) and baseline tricuspid E/A ratio (standardized $\beta=0.40$, $p=0.002$). Higher baseline sACE2 activity also modestly predicted a reduction in tricuspid E/A ratio over 72 hours (Spearman's $r=-0.32$, $p=0.050$). However, baseline or percentage change in sACE2 activity over 72 hours did not correlate with any other percentage changes in hemodynamic, echocardiographic or plasma BNP levels ($p>0.06$ for all).

Prognostic value of serum sACE2 activity changes before and after intensive medical treatment

In our study cohort, only 57 patients had follow-up sACE2 activity levels measured and therefore changes in sACE2 activity assessed. In this subgroup, 33 patients experienced the composite adverse event endpoint of all-cause mortality, cardiac transplantation, or HF re-hospitalization. Over the course of 48–72 hours of intensive medical therapy, there was a significant increase in serum sACE2 levels from baseline ($p=0.002$). Specifically, a 50% increase in baseline serum sACE2 levels during intensive medical treatment predicted a significantly reduced risk of death, cardiac transplantation or first HF re-hospitalization even after adjustment for baseline age, RAVi, and plasma BNP levels (Hazard ratio 0.35, 95%CI 0.12–0.84, $p=0.018$) (Table 3). Kaplan-Meier survival plots show significant increased event-free survival percentage in the patients with 50% increase of serum sACE2 after intensive medical treatment ($p=0.021$) (Figure 2). Interestingly, in the subgroup of 55 patients who had follow-up BNP levels assessed, serial changes in BNP did not demonstrate prognostic value in our cohort of patients with acute decompensated heart failure ($p=0.96$).

DISCUSSION

Increased sACE2 activity in patients with chronic HF has been associated with greater severity of HF symptoms, cardiac dysfunction, as well as more adverse long-term outcomes[5–7]. Our current study extends these findings into the ADHF setting and demonstrates that the ability to augment serum sACE2 following intensive medical therapy

in patients with ADHF may provide potential benefits in the improvement of beneficial counter-regulatory responses. These hypothesis-generating results are consistent with our mechanistic understanding of the benefits of restoring vascular homeostasis through augmentation of counter-regulatory actions and cardioprotective effects of ACE2[8–9].

Angiotensin converting enzyme 2 mRNA is present in all human tissues with high expression in the heart, kidney and gastrointestinal tissues, suggesting it plays key regulatory functions [10]. Within the heart, multiple cell types express ACE2, including endothelial cells, vascular smooth muscle cells as well as in cardiomyocytes, macrophages, and myofibroblasts[1–2, 11–12]. Aged ACE2-knockout mice displayed a severe cardiac contractility defect and increased Ang II levels[13]. Genetic ablation of ACE on ACE2 mutant background completely rescued the cardiac phenotype and reversed the increased Ang II levels, suggesting ACE and ACE2 have counter-balancing functions in the heart[13]. In response to myocardial infarction, ACE2-knockout mice displayed increased mortality, infarct expansion, and adverse ventricular remodeling characterized by ventricular dilation and systolic dysfunction[14]. Furthermore, ACE2 null mice were found to develop a progressive age-dependent dilated cardiomyopathy with pathological myocardial hypertrophy and fibrosis, and hypoxia-induced collagen production by cardiac fibroblasts can be inhibited by ACE2 overexpression[15]. These studies defined the critical role of ACE2 up-regulation as a compensatory response to HF, and the consequent increase of vasodilatory Ang 1–7 may confer cardioprotective effects to counterbalance the effects of Ang II. Our study demonstrated baseline sACE2 activity levels correlated positively with plasma BNP levels and this was still the case after 48-72-hour intensive medical treatment. This relationship provided an objective validation of the association between the systemic release of ACE2 and HF severity, which was similar to that reported previously[5–7]. Interestingly, the relationship between sACE2 activity and echocardiographic indices slightly differ between the acute and chronic setting[7], which is likely due to the advanced and decompensated nature of ADHF, where vascular and fluid homeostasis were altered.

The major finding of this study is that an increase in serum sACE2 activity levels after treatment, rather than baseline sACE2 activity levels at one time point, predicted better clinical outcomes in ADHF patients. Therapy that can increase circulating sACE2 activity may be beneficial for the long-term outcome of ADHF patients. Several studies noted a marked increase in cardiac ACE2 expression in response to ACE inhibitors[12], angiotensin receptor blockers[16], or mineralocorticoid receptor antagonists[17] in animal models, but others have failed to show such beneficial effects[18]. To our knowledge, this is the first report of serial changes in serum sACE2 activity levels in patients with HF with treatment and demonstrable therapeutic responses, and will therefore require confirmation in future human studies. Indeed, there have been experimental evidence to support the potential for increasing ACE2 activity in preventing heightened Ang II actions and protecting cardiovascular remodeling through an increase in cardiac nitric oxide production as well as attenuation of oxidative stress[16, 1920]. These findings are certainly consistent with the therapeutic interdictions prescribed during intensive medical therapy with the primary goal to improve hemodynamic derangements and titrate neurohormonal antagonists in the setting of advanced ADHF.

There are several limitations in this study that warrant discussion. First, we have to acknowledge that this is a subset of patients with advanced systolic HF undergoing hemodynamic-guided intensive medical therapy at the clinician's discretion, and therefore such changes may not directly translate to the broad ADHF population. Although improvement in serum sACE2 activity levels was associated with better outcomes, we cannot reliably confirm the specificity of circulating sACE2 activity and cardiovascular function especially when the correlations were modest. We also did not have antecedent

sACE2 activity levels to imply diminished sACE2 activity leading to HF decompensation. It is conceivable not only treatment effects but also restoration of other organ function (such as the kidneys[21]) may also play a role in improving sACE2 activity and associated favorable outcomes. Nevertheless, our findings were promising and support further investigations in targeting the ACE2-Ang1-7-Mas system to restore cardiovascular homeostasis. Indeed, recombinant ACE2 is already in clinical development[22–23], and our findings support the potential benefits for modulating renin-angiotensin-aldosterone system via exogenous administration. Based on these observations and the intrinsic understanding of the role ACE2 plays, we believe that while sicker patients with heart failure have higher levels in response to disease severity, the relatively diminished sACE2 activity in the acute decompensated setting signifies a potentially insufficient compensatory response to maintain clinical stability.

CONCLUSIONS

In patients admitted with ADHF, baseline serum sACE2 activity levels are modestly associated with RV diastolic dysfunction and plasma BNP levels, while increasing serum sACE2 activity levels during intensive medical therapy predict improved outcomes independent of underlying cardiac indices. These hypothesis-generating observations may warrant future validation.

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Figure 1.

Comparison of echocardiographic indices of right atrial volume index (RAVi in Figure 2A) and plasma B-type natriuretic peptide (BNP in Figure 2B) according to baseline sACE2 activity tertiles. 1st sACE2 Tertile: <25 ng/mL; 2nd sACE2 Tertile: 25–37 ng/mL; 3rd Tertile: 37 ng/mL. Abbreviations: sACE2, soluble angiotensin-converting enzyme 2; RAVi, right atrial volume index; BNP, B-type natriuretic peptide.

Figure 2.

Kaplan-Meier analysis of event-free survival stratified by percentage change in sACE2 activity before and after intensive medical treatment. *Abbreviations:* sACE2, soluble angiotensin-converting enzyme 2.

Table 1

Baseline Subject Characteristics (n=70).

Demographics	
Age (years)	56 ± 13
Male, n (%)	55 (79%)
African American, n (%)	10 (14%)
BMI (kg/m ²)	27 [24–30]
Heart failure history	
NYHA class, n (%)	III 25 (42%)
	IV 31 (53%)
Ischemic etiology, n (%)	37 (53%)
Co-morbidities	
Hypertension, n (%)	29 (41%)
Diabetes mellitus, n (%)	25 (36%)
Preadmission Medications	
ACEi and/or ARB, n (%)	20 (29%)
Beta-blocker, n (%)	31 (44%)
Aldosterone antagonist, n (%)	20 (29%)
Loop diuretics, n (%)	31 (44%)
Digoxin, n (%)	21 (30%)
Admission Medications	
ACEi and/or ARB, n (%)	19 (27%)
Beta-blocker, n (%)	20 (29%)
Aldosterone antagonist, n (%)	14 (20%)
Loop or thiazide diuretic, n (%)	42 (60%)
Inotrope, n (%)	29 (41%)
Vasodilator, n (%)	61 (87%)
Echocardiographic indices	
LV ejection fraction (%)	27 ± 9
LV end-diastolic volume index (mL/m ²)	110 ± 45
Diastolic stage III, n(%)	58 (83%)
Hemodynamic indices	
Mean PAP (mmHg)	31 ± 8
PCWP (mmHg)	21 ± 7
CVP (mmHg)	13 ± 6
CI (L/min/1.73m ²)	2.1 ± 0.7
Laboratory data	
sACE2 at baseline (ng/mL)	32 [23, 43]
sACE2 at 48–72hrs (ng/mL)	40 [28, 60]

eGFR (mL/min/1.73 m ²)	62 [39, 99]
BNP (pg/mL)	1412 [668, 2172]

Abbreviations: BMI, Body mass index; NYHA, New York Heart Association; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker, LV, left ventricular; PAP, pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; CVP, central venous pressure; CI, cardiac index; sACE2, soluble angiotensin-converting enzyme 2; eGFR, estimated glomerular filtration rate; BNP, B-type natriuretic peptide.

Table 2

Correlation between Baseline Serum sACE2 Levels and Baseline Hemodynamic, Echocardiographic, and Plasma BNP Levels

	Baseline sACE2 (ng/mL)	
	Spearman's r	p-value
Age (years)	0.03	0.800
BMI (kg/m ²)	-0.15	0.221
eGFR (mL/min/1.73m ²)	0.06	0.643
BNP (pg/mL)	0.32	0.008
Echocardiographic indices		
LV structure		
LV mass index (g/m ²)	-0.12	0.336
LV end-diastolic volume index (mL/m ²)	0.00	0.982
LV systolic function		
LV ejection fraction (%)	-0.05	0.670
LV diastolic function		
Mitral E/A ratio	0.07	0.617
Mitral DT (ms)	-0.13	0.288
Mitral E/Ea ratio	0.04	0.776
LA volume index (mL/m ²)	0.11	0.362
RV structure		
RV end-diastolic area (cm ²)	0.26	0.045
RV systolic function		
RV fractional area change (%)	-0.13	0.316
RV diastolic function		
Tricuspid E/A ratio	0.39	0.007
Tricuspid DT (ms)	0.02	0.914
Tricuspid E/Ea ratio	0.05	0.697
RA volume index (mL/m ²)	0.31	0.010
Hemodynamic indices		
Systolic PAP (mmHg)	-0.01	0.932
Diastolic PAP (mmHg)	-0.05	0.663
Mean PAP (mmHg)	0.00	0.998
PCWP (mmHg)	0.12	0.335
CVP (mmHg)	0.19	0.127
CI (L/min/m ²)	-0.16	0.189
SVR (dyn-s / cm ⁵)	0.29	0.017
PVR (dyn-s / cm ⁵)	-0.06	0.694

Abbreviations: sACE2, soluble angiotensin-converting enzyme 2; BMI, Body mass index; eGFR, estimated glomerular filtration rate; BNP, B-type natriuretic peptide; LV, left ventricular; DT, deceleration time; LA, left atrial; RV, right ventricular; RA, right atrial; PAP, pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; CVP, central venous pressure; CI, cardiac index; SVR, systemic vascular resistance; PVR, pulmonary vascular resistance.

Table 3

Cox proportional hazard analyses of adverse clinical outcomes (33 events).

Variable	HR (95% CI)	P value
sACE2 50%	0.34 (0.12 – 0.82)	0.014
Multivariable Model		
sACE2 50%	0.35 (0.12 – 0.84)	0.018
Age (years) *	0.81 (0.60 – 1.08)	0.154
RAVi (mL/m ²) *	1.16 (0.74 – 1.71)	0.509
Ln BNP (pg/mL) *	1.04 (0.73 – 1.50)	0.841

Abbreviations: HR, hazard ratio; CI, confidence interval; RAVi, right atrial volume index; BNP, B-type natriuretic peptide.

* HRs per 1 SD (SDs: Age = 13 yrs, RAVi = 19 mL/m², Ln BNP = 0.93 pg/mL)