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1 **Circulating plasma concentrations of ACE2 in men and women with heart failure and**
2 **effects of renin-angiotensin-aldosterone-inhibitors**

3

4 Izhiah E. Sama, PhD¹; Alice Ravera, MD^{1,2}; Bernadet T. Santema, MD¹; Harry van Goor,
5 PhD³; Jozine, M. ter Maaten, MD PhD¹; John G.F. Cleland MD⁴, Michiel Rienstra, MD PhD¹,
6 Alex W. Friedrich, MD⁵, Nilesh J. Samani MD, PhD⁶ ; Leong L. Ng MD, PhD⁶ ; Kenneth
7 Dickstein MD^{7,8} Chim C. Lang MD⁹; Gerasimos Filippatos MD^{10,11}; Stefan D. Anker MD,
8 PhD^{12,13}; Piotr Ponikowski MD¹⁴; Marco Metra, MD²,Dirk J. van Veldhuisen, MD PhD¹;
9 Adriaan A. Voors, MD PhD¹

10

11 ¹Department of Cardiology, University of Groningen, University Medical Center Groningen,
12 Groningen, The Netherlands;² Cardiology, Department of Medical and Surgical Specialties,
13 Radiologic Sciences and Public Health, University of Brescia, Brescia, Italy;³Department of
14 Pathology and Medical Biology, University of Groningen, University Medical Center
15 Groningen, Groningen, The Netherlands; ⁴Director of the Robertson Centre for Biostatistics &
16 Clinical Trials Unit, University of Glasgow and Professor of Clinical Cardiology, National
17 Heart & Lung Institute, Imperial College, London, UK;⁵Department of Medical
18 Microbiology, University of Groningen, University Medical Center Groningen, Groningen,
19 The Netherlands; ⁶Department of Cardiovascular Sciences, University of Leicester, Glenfield
20 Hospital, and NIHR Leicester Biomedical Research Centre, UK;⁷University of Bergen,
21 Bergen, Norway. ⁸Stavanger University Hospital, Stavanger, Norway; ⁹Division of Molecular
22 and Clinical Medicine, School of Medicine, University of Dundee Ninewells Hospital and
23 Medical School, Dundee, UK; ¹⁰ National and Kapodistrian University of Athens, School of

1 Medicine, Athens, Greece; ¹¹ University of Cyprus, School of Medicine, Nicosia, Cyprus;
2 ¹²Department of Cardiology (CVK) and Berlin Institute of Health Center for Regenerative
3 Therapies (BCRT), Germany; ¹³German Centre for Cardiovascular Research (DZHK) partner
4 site Berlin, Charité Universitätsmedizin Berlin, Germany; ¹⁴Department of Heart Diseases,
5 Medical University, Military Hospital, Wrocław, Poland.

6 **Address for correspondence**

7 Prof. Dr. A.A. Voors

8 Department of Cardiology, University Medical Center Groningen

9 Hanzeplein 1, 9713 GZ, Groningen, The Netherlands

10 Tel: +31 (0)50 3616161; Fax : +31 (0)50 3618062; e-mail: a.a.voors@umcg.nl

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1 **Abstract**

2 **Aims:** The current pandemic coronavirus SARS-CoV-2 infects a wide age-group but
3 predominantly elderly individuals, especially men and those with cardiovascular disease.
4 Recent reports suggest an association with use of renin-angiotensin-aldosterone system
5 (RAAS) inhibitors. Angiotensin-converting enzyme 2 (ACE2) is a functional receptor for
6 coronaviruses. Higher ACE2 concentrations might lead to increased vulnerability to SARS-
7 CoV-2 in patients on RAAS-inhibitors.

8 **Methods:** We measured ACE2 concentrations in 1485 men and 537 women with heart failure
9 (Index cohort). Results were validated in 1123 men and 575 women (Validation cohort).

10 **Results:** The median age was 69 years for men, and 75 years for women. The strongest
11 predictor of elevated concentrations of ACE2 in both cohorts was male sex (estimate=0.26,
12 $p<0.001$ and 0.19; $p<0.001$ respectively). In the index cohort, use of ACE-inhibitors,
13 angiotensin receptor blockers (ARBs) or mineralocorticoid receptor antagonists (MRAs) were
14 not independent predictors of plasma ACE2. In the validation cohort, ACE-inhibitor
15 (estimate=-0.17; $p=0.002$) and ARB use (estimate=-0.15; $p=0.03$) were independent
16 predictors of lower plasma ACE2, while use of a MRA (estimate=0.11; $p=0.04$) was an
17 independent predictor of higher plasma ACE2 concentrations.

18 **Conclusion:** In two independent cohorts of patients with heart failure, plasma concentrations
19 of ACE2 were higher in men than women, but neither use of an ACE-inhibitor nor an ARB
20 was associated with higher plasma ACE2 concentrations. These data might explain the higher
21 incidence and fatality rate of COVID-19 in men, but do not support previous reports
22 suggesting that ACE-inhibitors or ARBs increase the vulnerability for COVID-19 through
23 increased plasma ACE2 concentrations.

1 **Keywords:** Men, Heart Failure, Coronavirus disease(COVID-19), ACE2

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1 **Introduction**

2 The world is currently faced with the outbreak of a new Severe Acute Respiratory Syndrome
3 coronavirus (SARS-CoV). The new virus, SARS-CoV-2 emerged in December 2019 in the
4 city of Wuhan China, and is the causative agent of a respiratory syndrome now known as
5 coronavirus disease 2019 (COVID-19)^{1, 2}. Efforts aimed at curbing the spread of SARS-CoV-
6 2 and finding effective treatments are ongoing.

7 Early epidemiological observations indicate that SARS-CoV-2 infects all age-groups, but
8 older men with chronic illnesses may be more severely affected. There is a preponderance of
9 men (58.1%) compared to women (41.9%) testing positive for COVID-19² and in the
10 previous SARS-CoV epidemic in 2003, men had a higher mortality than women (21.9%
11 versus 13.2%; $p < 0.0001$)³. Whether men with the current SARS-CoV-2 virus also have a
12 worse mortality outcome is becoming apparent as recent report indicate that 70% of patients
13 that died of COVID-19 in Italy were men⁴; and mainly elderly.

14 The increased vulnerability of older people with cardiovascular disease and comorbid
15 conditions could be related to increased concentrations of angiotensin-converting enzyme 2
16 (ACE2)^{5, 6}, and ACE2 is known to be increased in heart failure⁷. ACE2 is not only an enzyme
17 but also a functional receptor on cell-surfaces for both SARS-CoV and SARS-CoV-2 and is
18 highly expressed in the heart, testis, kidneys and lungs⁸⁻¹² and shed into the plasma. Some
19 reports have suggested that inhibitors of the renin-angiotensin-aldosterone system (RAAS)
20 increase plasma ACE2 concentrations^{5, 13}, although these speculations are not supported by a
21 substantial body of research.

22 We therefore investigated plasma concentrations of ACE2 in two large and independent
23 cohorts of men and women with heart failure according to the use of renin-angiotensin-
24 aldosterone-inhibitors.

1 **Methods**

2 **Study participants**

3 From the BIOlogy Study to TAIlored Treatment in Chronic Heart Failure (BIOSTAT-CHF)
4 ¹⁴, we measured ACE2 concentrations in 1485 men and 537 women with heart failure in 11
5 European countries. Results were validated in another, independent BIOSTAT-CHF cohort
6 consisting of 1123 men and 575 women with heart failure enrolled in Scotland. Only
7 participants with sufficient plasma samples were used for this research. The design and
8 baseline characteristics of both cohorts of BIOSTAT-CHF have been published elsewhere¹⁴.
9 Inclusion criteria were the same in the index and validation cohorts; the only exception was
10 that when the left ventricular ejection fraction (LVEF) was > 40%, patients had to have a
11 BNP >400ng/L or NT-proBNP >2,000ng/L in the index cohort, but not in the validation
12 cohort. The study complied with the Declaration of Helsinki and was approved by the medical
13 ethics committees of participating centers¹⁴.

14 ACE2 was measured using the Olink Proseek analysis service (Olink Proteomics, Uppsala,
15 Sweden). The Olink platform¹⁵ utilizes a high-throughput multiplex immunoassay based on a
16 proprietary Proximity Extension Assay (PEA) technology, where each biomarker is addressed
17 by a matched pair of antibodies, coupled to unique, partially complementary oligonucleotides,
18 and measured by quantitative real-time polymerase chain reaction (PCR). Results are
19 expressed in the form of relative quantification (Normalized Protein eXpression or NPX)
20 which are logarithmically related to protein concentration but cannot be converted to absolute
21 protein concentrations. Interpretations are therefore relative and not absolute. Analytical
22 validation of the sensitivity and specificity of the Olink assay for this study was achieved by
23 comparing available routine laboratory measurements of two protein biomarkers, GDF15

1 (pg/mL) and NT-proBNP (pg/mL) with those measured using Olink (NPX). NT-proBNP is a
2 canonical biomarker in cardiovascular disease biology¹⁶.

3 **Statistical Analyses**

4 All statistical analyses were performed using R¹⁷ version 3.6.2. In group comparisons,
5 categorical variables were depicted as numbers with percentages. Normally distributed
6 variables were depicted as means \pm standard deviation, non-normally distributed variables as
7 median and interquartile range (IQR) defined as the first and third quartile (Q1–Q3). The
8 means for continuous variables were compared by one-way analysis of variance (ANOVA) or
9 the Kruskal–Wallis test, while categorical variables were compared by the Chi-squared test.
10 Multivariate models were based on backward stepwise regression. Baseline tables were made
11 using the R-based CompareGroups¹⁸ package. In general, a two-tailed p-value of <0.05 was
12 considered statistically significant.

13

14 **Results**

15 **Clinical characteristics**

16 Baseline characteristics of the index and validation cohort are presented in table 1 and
17 supplemental table 1 respectively. In the index cohort (n=2022), the median age was 69 years
18 in men (interquartile range, IQR, 60-76), and for women 75 years (IQR 64-81; $p<0001$
19 between men and women). In the validation cohort (n=1698), the median age for men was 74
20 years (IQR 66-81) and for women 76 years (IQR 69-82; $p< 0.001$ between men and women).
21 In the index cohort, patients with higher concentrations of ACE2 were more often men, were
22 more likely to have atrial fibrillation and a higher heart rate and lower systolic blood pressure
23 (table 1). In the validation cohort, patients with higher concentrations of ACE2 were more

1 often men, were more likely to have atrial fibrillation and diabetes and a higher heart rate and
2 lower systolic blood pressure (supplemental table 1). In the index cohort, only 0.3% (6/2022)
3 patients received both ACEi and ARB. In the validation cohort only 0.4% (7/1691) received
4 both ACEi and ARB.

5 Among patients that were not treated with RAAS-inhibitors, men were predominant in the
6 uppermost quartile of ACE2 (supplemental table 2 and 3). ACE2 concentrations were higher
7 in men than women in 9/11 countries but were similar by ACEi/ARB use (Supplemental
8 Figure 1 and 2).

9 **Analytical validation of the Olink assay**

10 In both study cohorts, routine lab concentrations of two golden standard biomarkers (GDF-15
11 and NT-proBNP) showed a strong correlation with those measured using Olink (Spearman's
12 rho 0.77-0.92, $p < 0.001$; Supplemental Figure 3).

13 **ACE2 concentrations in men and women according to the use of RAAS-inhibitors**

14 The ACE2-RAAS-COVID-19 axis is summarized in Figure 1. In both cohorts, plasma ACE2
15 concentrations (in NPX units) were higher in men than in women. In the index cohort, mean
16 plasma concentration was 5.38 in men compared with 5.09 in women ($p < 0.001$). In the
17 validation cohort, mean plasma concentration was 5.46 in men compared with 5.16 in women
18 ($p < 0.001$).

19 Figure 2 shows plasma ACE2 concentrations in those treated with or without blockers of the
20 renin-angiotensin-aldosterone system. In the index cohort, mean plasma concentration was
21 5.32 in patients who used an ACE-inhibitor compared with 5.29 in those who did not ($p =$
22 0.59). In the validation cohort, mean plasma concentration was 5.32 in those who used an
23 ACE-inhibitor versus 5.4 in those who did not ($p = 0.0033$). In the index cohort, mean plasma

1 concentration was 5.23 in patients who used an ARB compared with 5.31 in those who did
2 not ($p = 0.16$). In the validation cohort, mean plasma concentration was 5.3 in those who used
3 an ARB versus 5.37 in those who did not ($p = 0.38$). In the index cohort, mean plasma
4 concentration was 5.35 in patients who used an MRA compared with 5.25 in those who did
5 not ($p = 0.003$). In the validation cohort, mean plasma concentration was 5.4 in those who
6 used an MRA versus 5.34 in those who did not ($p = 0.036$).

7 Age and sex interaction analyses indicated that men who used MRA have an increased ACE2
8 concentration ($p \leq 0.01$, for models unadjusted, and those adjusted for ACEi use, ARB use,
9 age, diabetes and atrial fibrillation). This was statistically significant only in the index cohort.
10 In the validation cohort, men who used ACEi had lower ACE2 concentrations ($p < 0.05$; for
11 models unadjusted, and those adjusted for ACEi use, ARB use, age, diabetes and atrial
12 fibrillation). All similar interaction tests were not statistically significant.

13 **Variables associated with plasma ACE2 concentrations**

14 The strongest predictor of elevated plasma concentrations of ACE2 in the index and
15 validation cohort was male sex (estimate=0.26, $p < 0.001$ and 0.19; $p < 0.001$ respectively). In
16 the index cohort, neither ACE-inhibitors, ARBs nor MRAs were associated with plasma
17 ACE2 concentrations (table 2). In the validation cohort, ACE-inhibitors (estimate=-0.17;
18 $p = 0.002$) and ARBs (estimate=-0.15; $p = 0.03$) were associated with lower plasma ACE2
19 concentrations, but MRAs (estimate=0.11; $p = 0.04$) were associated with higher concentrations
20 (supplemental table 4).

21

22 **Discussion**

1 In two large independent cohorts of patients with heart failure, we found that plasma ACE2
2 concentrations were higher in men than in women. In addition, those receiving ACE-
3 inhibitors or ARBs did not have higher concentrations of ACE2 and an increase in those
4 taking MRA in the validation cohort was not confirmed in the index cohort.

5 There is an increased susceptibility of elderly people with chronic comorbidities to SARS
6 coronaviruses and men appear to be especially vulnerable to SARS-CoV-2^{1, 2, 19}. Given that a
7 typical heart failure patient belongs to this high-risk group, we sought to uncover factors that
8 could explain the sex-based susceptibility to SARS-CoV-2 in this vulnerable population.

9 Baseline characteristics of the two cohorts presented are typical for patients with heart failure
10 and confirm that these are elderly patients that often have comorbidities, including diabetes,
11 hypertension, renal disease and COPD. The spectrum of comorbidities involves most of the
12 organs affected in COVID-19, including the heart, lungs, kidneys and liver¹.

13 COVID-19 patients and other patients with such underlying diseases are in a hyper-
14 inflammatory state. As such it might well be that patients with various kidney diseases have
15 high endothelial ACE2²⁰; making ACE2 a damage marker. Furthermore, plasma ACE2
16 activity is increased in patients with heart failure²¹.

17 **Post-transcriptional events of ACE2 in the testis**

18 ACE2 is widely distributed in tissues including lung alveolar epithelial cells, vascular
19 endothelium, heart, kidney and testis^{8, 11, 20, 22}. For the readers' convenience, we provide
20 Supplemental Figures 4 and 5 showing the gene structure of ACE2, and its isoforms and
21 tissue distribution). ACE2 protein and interestingly also the non-coding isoforms are highly
22 expressed in the testis²² (Supplemental Figure 6). Isoform transcription could possibly affect
23 protein translation in this male-specific tissue; for example via MicroRNA (miRNA)

1 competition. Previous studies indicate that ACE2 may be subject to post-transcriptional
2 regulation via miR-421²³ which could be exploited as a novel potential therapeutic target to
3 modulate ACE2 expression in disease. How the testis-expressed ACE2 protein, or those
4 expressed at other organs, enters circulation is largely unknown. The tissue-specific
5 transcriptional regulation of ACE2 could partially explain higher ACE2 protein
6 concentrations and why a coronavirus would flourish in men.

7 **ACE2 plasma concentrations and use of RAAS-inhibitors**

8 Recently, it was suggested that the higher prevalence and fatality rate in patients with cardiac
9 diseases, such as hypertension or diabetes, was related to the concomitant use of ACE-
10 inhibitors and ARBs that were suggested to increase ACE2 concentrations. The authors
11 speculated that this might be due to increased expression of ACE2 but offered no evidence for
12 this^{5, 6, 24, 25}. In animal models, selective blockade of either Angiotensin II synthesis or activity
13 induced increases in cardiac ACE2 gene expression and cardiac ACE2 activity¹³, whether this
14 translates to humans needs to be validated.

15 To the best of our knowledge, this is the first substantial study to examine the association
16 between plasma ACE2 concentrations and the use of RAAS-blockers in patients with
17 cardiovascular disease. In contrast to previous reports^{5, 6, 24, 25}, ACE-inhibitors and ARBs were
18 not associated with increased plasma concentrations of ACE2 in the present study. Indeed, if
19 anything, the use of ACE-inhibitors and ARB predicted *lower* concentrations of ACE2 in the
20 validation cohort, although these findings were not replicated in the index cohort. Taken
21 together, these data do not support withholding ACE-inhibitors or ARBs in patients at risk for
22 SARS-CoV-2 infection.

1 In the validation cohort, MRA use was associated with a weak but statistically significant
2 increase in plasma ACE2 concentrations. Univariate and multivariate-adjusted analyses
3 indicated a significant sex-based interaction; with men on MRA having higher ACE2
4 concentrations. A similar association was, not found in the index cohort. The effect of MRA
5 on plasma ACE2 is therefore not clear. One study found a trend ($p=0.07$) towards increased
6 plasma ACE2 activity in patients treated with an MRA²¹. In addition, one mechanistic study
7 using macrophages reported an increase of ACE2 activity after MRA²⁶ but further data are not
8 available. Clearly, our findings do not suggest that MRAs should be discontinued in patients
9 with heart failure, in whom coronavirus SARS-CoV-2 infection is found. Moreover, even if
10 MRAs are consistently found to increase plasma ACE2 concentrations, it still needs to be
11 established whether their use is associated with higher vulnerability to or more severe
12 consequences of SARS-CoV-2 infection. MRAs are a very effective treatment for heart
13 failure and these hypothetical effects on viral infection should be weighed carefully against
14 their proven benefits.

15 The equilibrium between soluble and membrane-bound ACE2 might influence COVID-19
16 pathogenesis and treatment options. Previous studies indicate ADAM17
17 mediated ACE2 shedding^{27, 28}, but how this would affect coronavirus infectivity during
18 concomitant use of RAAS inhibitors warrants a separate research. A study on dogs with heart
19 disease indicated that ACE2 shedding is not an important factor in the total extent of
20 tissue-bound ACE2 activity, but rather a loss of tissue ACE2 into the circulation would tend
21 to decrease the overall compensatory potential of ACE2²⁹. Further work is required to show
22 whether this translates to humans.

23

24 **Conclusion:**

1 In two large cohorts of patients with heart failure, plasma ACE2 concentrations were higher in
2 men than in women, possibly reflecting higher tissue expression of this receptor for SARS
3 coronavirus infections. This could explain why men might be more susceptible to infection
4 with, or the consequences of, SARS-CoV-2. Patients receiving ACE-inhibitors or ARBs did
5 not have higher plasma concentrations of ACE2 and any effect of MRA was small and
6 inconsistent, supporting the continued use of these agents in patients with heart failure during
7 the current SARS-CoV-2 pandemic.

8

9 **Limitations**

10 The conclusions drawn in this analysis are mainly restricted to heart failure, albeit a group of
11 patients at high risk for COVID-19. Secondly, since our patients are not coronavirus-infected,
12 we cannot provide a direct link between the course of COVID-19 disease in patients with low
13 versus high plasma ACE2 concentrations, and the influence of age and RAAS-blockers on the
14 course of the disease. Thirdly, we measured plasma ACE2 concentrations and not membrane-
15 bound ACE2. We can only speculate that circulating concentrations are associated with tissue
16 concentration, since there is no compelling evidence for this.

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1 **Figure Legend**

2 **Figure 1:** Summary of the RAAS pathway

3 **Figure 2: ACE2 concentrations in patients with and without use of ACEi, ARB and**
4 **MRA (index and validation).**

5 ACEi = Use of Angiotensin Converting Enzyme (ACE) inhibitors; ARB = use of Angiotensin
6 Receptor Blocker; MRA = use of Mineralocorticoid Receptor Antagonist.

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8 **Tables**

9 **Table 1:** Baseline characteristics according to quartiles of plasma ACE2 concentrations
10 (index cohort)

11 **Table 2:** Multivariable predictors of plasma ACE2 concentrations (index cohort)

12 Supplemental Figure 1: Global perspective of BIOSTAT-CHF countries and mean ACE2
13 levels

14 Supplemental Figure 2: Global impression of ACE2 levels in men and women, and upon use
15 of ACEi/ARB per country studied

16 Supplemental Figure 3: Analytical validation of Olink assay using routine lab measurements
17 of GDF-15 and NT-proBNP

18 Supplemental Figure 4: The ACE2 gene model and exons encoding domains. (Coronavirus S1
19 proteins binds to the N-terminal peptidase domain of ACE2 (Source: the Ensembl genome
20 browser))

1 Supplemental Figure 5: Isoforms and tissue distribution of ACE2. (Data Source: GTEx
2 Analysis Release V8 (dbGaP Accession phs000424.v8.p2)

3 Supplemental Figure 6: Co-expression of ADAM17 and ACE2 at the testis and other tissues
4 (left=RNA level; right=protein level. (Source Human Protein Atlas))

5 Supplemental Table 1: Baseline characteristics according to quartiles of plasma ACE2
6 concentrations (validation cohort)

7 Supplemental Table 2: Baseline characteristics according to quartiles of plasma ACE2
8 concentrations for patients not on RAAS-inhibitors (index cohort)

9 Supplemental Table 3: Baseline characteristics according to quartiles of plasma ACE2
10 concentrations for patients not on RAAS-inhibitors (validation cohort)

11 Supplemental Table 4: Multivariable predictors of plasma ACE2 concentrations (validation
12 cohort)

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