



# Angiotensin-converting enzyme 2 (ACE2) levels in relation to risk factors for COVID-19 in two large cohorts of patients with atrial fibrillation

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## Aims

The global COVID-19 pandemic is caused by the SARS-CoV-2 virus entering human cells using angiotensin-converting enzyme 2 (ACE2) as a cell surface receptor. ACE2 is shed to the circulation, and a higher plasma level of soluble ACE2 (sACE2) might reflect a higher cellular expression of ACE2. The present study explored the associations between sACE2 and clinical factors, cardiovascular biomarkers, and genetic variability.

## Methods and results

Plasma and DNA samples were obtained from two international cohorts of elderly patients with atrial fibrillation ( $n = 3999$  and  $n = 1088$ ). The sACE2 protein level was measured by the Olink Proteomics<sup>®</sup> Multiplex CVD II<sup>96 × 96</sup> panel. Levels of the biomarkers high-sensitive cardiac troponin T (hs-cTnT), N-terminal probrain natriuretic peptide (NT-proBNP), growth differentiation factor 15 (GDF-15), C-reactive protein, interleukin-6, D-dimer, and cystatin-C were determined by immunoassays. Genome-wide association studies were performed by Illumina chips. Higher levels of sACE2 were statistically significantly associated with male sex, cardiovascular disease, diabetes, and older age. The sACE2 level was most strongly associated with the levels of GDF-15, NT-proBNP, and hs-cTnT. When adjusting for these biomarkers, only male sex remained associated with sACE2. We found no statistically significant genetic regulation of the sACE2 level.

## Conclusions

Male sex and clinical or biomarker indicators of biological ageing, cardiovascular disease, and diabetes are associated with higher sACE2 levels. The levels of GDF-15 and NT-proBNP, which are associated both with the sACE2 level and a higher risk for mortality and cardiovascular disease, might contribute to better identification of risk for severe COVID-19 infection.

## Keywords

COVID-19 • ACE2 • Biomarker • Cardiovascular disease • Atrial fibrillation • Age

## Introduction

The rapidly spreading COVID-19 pandemic<sup>1–3</sup> caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)<sup>3,4</sup> invades mainly cells in the respiratory tract, which might lead to a life-threatening pneumonitis.<sup>5</sup> The most recent pandemic with the H1N1

influenza virus mainly affected young adults without any sex difference.<sup>6,7</sup> In contrast, COVID-19 is more severe at older age, with comorbidities, i.e. diabetes mellitus, hypertension, and vascular disease, and in men.<sup>1,8–10</sup>

The SARS-CoV-2 virus adheres to the pulmonary alveolar epithelial cells by its surface spike protein, which binds to the cell

membrane-bound angiotensin-converting enzyme 2 (ACE2).<sup>5,11–13</sup> In this context ACE2 functions as a receptor following activation of the SARS-CoV-2 spike protein by the cellular transmembrane protease serine 2 (TMPRSS2).<sup>14</sup> After entering the cell, the SARS-CoV-2 virus replicates rapidly, leading to high early virus loads.<sup>15</sup> The soluble ACE2 (sACE2) protein is shed into the circulation.<sup>14,16,17</sup> In healthy subjects and patients with stable chronic diseases, the sACE2 level probably reflects the expression and availability of ACE2 at the cellular level. Currently there is limited knowledge on the regulation of ACE2 expression and circulating sACE2 levels in humans. Recently we measured the levels of sACE2 protein in plasma, as part of a project on multiplex proteomics in relations to outcomes in two large global cohorts of patients with atrial fibrillation (AF). Now we have taken the opportunity to explore the levels of sACE2 and their relationship to risk factors for COVID-19 and genetic variability in these two cohorts. The extended knowledge on the interplay between sACE2 levels and clinical manifestations of biomarkers reflecting cardiovascular dysfunction, might improve the understanding of the higher risk of COVID-19 in males and the elderly with cardiovascular disease.

## Materials and methods

### Cohorts

The ARISTOTLE trial included 18 201 patients with AF and an increased risk of stroke who were randomized to a median of 1.9 years treatment with warfarin or apixaban. Biomarker samples at baseline were available from 14 611 participants and, for economic reasons, a random sample of 3999 patients was used for the multiplex proteomics analysis including the sACE2 measurements in the current study. The RE-LY trial included 18 113 patients with AF and an increased risk of stroke to a median of 2.0 years treatment with dabigatran or warfarin. Biomarker samples drawn at baseline were available in 8548, out of which a random sample of 1088 was used for the multiplex proteomics analysis including the sACE2 measurements for this study. The details of the trials and the biomarker programmes have previously been published.<sup>18–20</sup> The trials complied with the Declaration of Helsinki and were approved by appropriate ethics committees at all sites. All patients provided written informed consent.

### Samples

In the ARISTOTLE<sup>19,20</sup> and the RE-LY<sup>18</sup> trials, patients provided blood samples at randomization into vacutainer tubes containing EDTA, which were centrifuged immediately. Plasma was pipetted into freezing tubes in aliquots and stored at  $-70^{\circ}\text{C}$  until analyses.

### Biochemical methods

The analysis of the ACE2 level in plasma was performed at the Clinical Biomarkers Facility, Science for Life Laboratory, Uppsala University, Uppsala, Sweden. The determination of the sACE2 protein level was performed using Proximity Extension Assay (PEA) with the Olink Proteomics<sup>®</sup> Multiplex CVDII<sup>96</sup> × <sup>96</sup> panel.<sup>21</sup> The plasma concentrations of high-sensitive cardiac troponin T (hs-cTnT), N-terminal probrain natriuretic peptide (NT-proBNP), and growth differentiation factor 15 (GDF-15) were determined by Roche immunoassays using a Cobas Analytics e601 (Roche Diagnostics, Penzberg, Germany).<sup>18–20</sup> High sensitive C-reactive protein (CRP) was analysed using a particle-enhanced immunoturbidimetric assay, (Abbott, Abbott Park, IL, USA) and high-

sensitivity interleukin-6 (IL-6) by sandwich ELISA immunoassays (R&D Systems Inc., Minneapolis, MN, USA). D-dimer was determined by an enzyme immunoassay (Asserachrome, Stago, France). Cystatin C was analysed with the ARCHITECT system ci8200 (Abbott Laboratories) using the particle-enhanced turbidimetric immunoassay (PETIA) from Gentian (Moss, Norway). Estimated glomerular filtration rate (eGFR) was calculated based on centrally determined creatinine levels using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. The conventional biomarker analyses were performed at the Uppsala Clinical Research Center Laboratory, Uppsala University, Uppsala, Sweden.

### Statistical methods

The associations between sACE2 and a number of pre-specified variables (Table 1) were analysed using linear regression models with sACE2 as outcome. As sACE2 is measured on the OLINK CVDII chip, the measuring unit is NPX, representing a log<sub>2</sub> scale of some original unit of the concentration. Thus, parameter estimates from the regression models should be interpreted as the difference in mean log<sub>2</sub> concentration of sACE2. Hence, an estimate of 1 would indicate a doubling of concentration on the unknown original scale.

We fitted unadjusted models including each of the variables of interest as well as multivariable models including (i) all 'clinical' variables (age, sex, body mass index, current smoker, hypertension, diabetes, prior myocardial infarction, prior coronary revascularization, prior stroke/transient ischaemic attack, prior peripheral arterial disease, congestive heart failure); (ii) all clinical variables and treatments at baseline (beta-blocker, aspirin, clopidogrel, statin, ACE inhibitor or angiotensin receptor blocker, amiodarone); and (iii) all clinical variables, treatments at baseline, and standard biomarkers (NT-proBNP, hs-cTnT, GDF-15, cystatin C, and IL-6).

All continuous variables were represented by restricted cubic splines with four knots placed at the marginal 5th, 35th, 65th, and 95th sample percentiles. For these variables, comparisons of the mean sACE2 were made for the difference between the third and the first sample quartile. Associations between sACE2 and each variable in the equations were presented graphically as predicted values with corresponding 95% confidence intervals, holding all other variables constant at the median value for continuous variables, 'male' for sex, and 'no' for prior diseases or treatments at baseline.

As there were 358 patients with missing values for CRP, IL-6, cystatin C, and D-dimer in RE-LY (Table 1), multiple imputation was used for the RE-LY data. The proportion of subjects with non-complete data was ~35%. Therefore, 35 imputed datasets were created with predictive mean matching using the mice function in the R add-on package mice.<sup>22</sup> Analyses were performed on the imputed datasets and combined using the function fit.mult.impute in the Hmisc<sup>23</sup> package. In a sensitivity analysis, all analyses were performed on the complete cases only in RE-LY, with the results materially unaltered (data not shown).

All analyses were done following the regression modelling strategies outlined by Harrell<sup>24</sup> using R (version 3.6.1).<sup>25</sup> In particular, the packages Hmisc and rms,<sup>23,24</sup> and mice<sup>22,26</sup> were used.

### Genetic methods

Participation in the ARISTOTLE and RE-LY genetic substudies with whole blood sampling for DNA extraction was voluntary and based on signing a separate consent form. The genotyping was performed in a subset of 5620 patients in ARISTOTLE using the Illumina Global Screening Array (24v2) and a subset of 3076 patients in RE-LY using the Illumina Human610-quad chip (Illumina, San Diego, CA, USA). Quality controls were performed using the whole-genome association analysis toolset PLINK v1.9 (<https://www.cog-genomics.org/plink/>)<sup>27</sup> [sex check, call rate

**Table 1** Baseline characteristics in the ARISTOTLE and RE-LY cohorts

Cohort	ARISTOTLE	RE-LY
Patients	<i>n</i> = 3999	<i>n</i> = 1088
Age, (years) median (IQR)	70.0 (63.0–76.0)	72.0 (67.0–77.0)
Sex: male	63.0% (2519)	62.8% (683)
Body mass index, kg/m <sup>2</sup>	28.6 (25.4–32.7) [19]	27.9 (24.9–31.2) [2]
Current smoker	8.8% (353)	7.5% (82)
Diabetes	25.0% (998)	21.1% (230)
Hypertension	87.7% (3506)	79.0% (859)
Congestive heart failure	31.2% (1247)	29.1% (317)
Prior myocardial infarction	13.0% (518)	16.8% (183)
Prior peripheral arterial disease	4.8% (193)	3.6% (39)
Prior stroke/TIA	18.4% (736)	20.3% (221)
Medications		
Prior beta-blocker	66.5% (2660)	61.4% (668)
Prior aspirin	41.7% (1667)	40.5% (441)
Prior alopido-grel	3.9% (154)	5.0% (54)
Prior statin	41.3% (1652)	42.2% (459)
Prior ACE inhibitor	50.9% (2036)	49.9% (543)
Prior ARB	23.8% (952)	21.2% (231)
Prior ACE inhibitor or ARB	71.6% (2864)	68.5% (745)
Prior amiodarone	13.5% (538)	12.9% (140)
Biomarkers		
NT-proBNP (ng/L)	697.5 (368.0–1248.8) [3]	845.5 (391.8–1470.0) [0]
hs-cTnT (ng/L)	10.8 (7.4–16.6)	12.1 (7.7–19.5)
GDF-15 (ng/L)	1371.0 (966.0–2060.0)	1474.5 (1097.5–2147.5)
CRP (mg/L)	2.2 (1.0–4.6) [4]	2.5 (1.2–5.5) [357]
IL-6 (ng/L)	2.3 (1.5–4.0) [1]	2.4 (1.5–3.9) [358]
eGFR (CKD-EPI) (mL/min)	56.3 (45.5–68.6) [1]	65.7 (54.9–76.9) [9]
Cystatin C (mg/L)	1.0 (0.8–1.2) [4]	1.0 (0.8–1.2) [357]
D-dimer (µg/L)	521.0 (332.0–859.0) [26]	511.5 (322.2–888.0) [358]
Soluble ACE2 (sACE2) (NPX)	3.9 (3.5–4.4)	4.4 (3.9–4.8)

TIA, transient ischaemic attack; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; NT-proBNP, N-terminal probrain natriuretic peptide; hs-cTnT, high-sensitive cardiac troponin T; GDF-15, growth differentiation factor 15; CRP, C-reactive protein; IL-6, interleukin-6; eGFR, estimated glomerular filtration rate; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration. Numbers in square brackets represent missing data.

single nucleotide polymorphism (SNP) and individual >98%, minor allele frequency (MAF) >0.001, and Hardy–Weinberg equilibrium (HWE) *P*-value >1 × 10<sup>-8</sup>. Imputation of genotypes was performed using the Haplotype reference consortium v1.1, utilizing Eagle v2.3.3 for phasing<sup>28</sup> and PBWT for imputation.<sup>29</sup> Post-imputation, the data were filtered (MAF >0.005 and IMPUTE2s Info metric >0.7) and converted to hard calls using Plink.<sup>27</sup> As the random sampling for sACE2 analyses did not consider availability to whole-genome association study (GWAS), there were only 1583 and 289 subjects included both in the subset with measurements of sACE2 and in the subset with performance of GWAS analyses in the ARISTOTLE and RE-LY cohorts, respectively. Analyses of the GWAS were performed using linear regression implemented in Plink v1.9,<sup>27</sup> adjusting for age, sex, and the first four genetic principal components. The X chromosome was modelled assuming inactivation of the X chromosome and assuming no inactivation of the X chromosome as a sensitivity analysis. Meta-analysis of the GWAS results was performed using a fixed effects model. The genetic results are reported on build 37 hg19.

## Results

The baseline characteristics were fairly similar in both cohorts, as summarized in Table 1. There were no relevant differences between the random cohorts used for this study and the total cohorts of patients included in the biomarker substudies of the two trials (Supplementary material online, Tables S3a and S4b and b). Both populations were elderly, with median ages of 70 and 72 years in ARISTOTLE and RE-LY, respectively. There were 73% male patients in both cohorts. Body mass index was high, with three-quarters of the patients overweight. Hypertension was the most common risk factor; 88% in ARISTOTLE and 79% in the RE-LY cohort. Congestive heart failure was observed in 31% and 29%, and diabetes in 25% and 21% of the respective populations. The use of medications was similar between the cohorts. The biomarker levels were slightly higher in the RE-LY than in the ARISTOTLE population. However, the results

**Table 2** Baseline characteristics in relation to quartile groups of sACE2 levels in the ARISTOTLE cohort

Soluble ACE2 (NPX)	(1.30–3.55)	(3.55–3.91)	(3.91–4.37)	(4.37–6.87)
Patients	(n = 1000)	(n = 1000)	(n = 1000)	(n = 999)
Age (years)	69.0 (61.8–75.0)	70.0 (62.8–76.0)	70.5 (63.0–77.0)	70.0 (63.0–76.0)
Gender: male	51.2% (512)	64.7% (647)	67.0% (670)	69.1% (691)
Race: Caucasian	84.7% (847)	84.1% (841)	83.9% (839)	82.3% (822)
Black	0.8% (8)	1.2% (12)	1.1% (11)	1.3% (13)
American Indian	0.2% (2)	0.4% (4)	0.3% (3)	0.0% (0)
Asian	13.5% (135)	12.7% (127)	13.7% (137)	14.7% (147)
Other	0.8% (8)	1.6% (16)	1.0% (10)	1.7% (17)
Body mass index, kg/m <sup>2</sup>	28.7 (25.4–33.1) [3]	28.7 (25.4–32.7) [4]	28.6 (25.4–32.3) [2]	28.4 (25.3–32.4) [10]
Current smoker	8.1% (81)	8.2% (82)	9.4% (94)	9.6% (96)
Diabetes	22.4% (224)	22.7% (227)	23.4% (234)	31.3% (313)
Hypertension	87.5% (875)	88.8% (888)	86.5% (865)	87.9% (878)
Congestive heart failure	26.2% (262)	29.7% (297)	31.8% (318)	37.0% (370)
Prior myocardial infarction	9.5% (95)	13.5% (135)	13.8% (138)	15.0% (150)
Prior peripheral arterial disease	3.9% (39)	4.8% (48)	5.3% (53)	5.3% (53)
Prior stroke/TIA	17.9% (179)	19.1% (191)	19.7% (197)	16.9% (169)
Beta-blocker	64.2% (642)	66.7% (667)	64.5% (645)	70.7% (706)
Aspirin	42.7% (427)	40.6% (406)	40.3% (403)	43.1% (431)
Clopidogrel	3.5% (35)	3.1% (31)	5.0% (50)	3.8% (38)
Statin	41.1% (411)	39.7% (397)	42.0% (420)	42.4% (424)
ACE inhibitor (ACEi)	47.9% (479)	52.2% (522)	50.0% (500)	53.6% (535)
Angiotensin receptor blocker (ARB)	22.7% (227)	23.9% (239)	22.4% (224)	26.2% (262)
ACEi or ARB	68.2% (682)	73.0% (730)	69.4% (694)	75.9% (758)
Amiodarone	17.1% (171)	13.2% (132)	12.2% (122)	11.3% (113)
NT-proBNP (ng/L)	538.0 (241.5–926.0) [1]	645.0 (336.5–1119.0) [1]	754.0 (418.0–1346.0) [1]	915.0 (479.0–1673.0) [0]
hs-cTnT (ng/L)	8.8 (6.3–13.1)	10.3 (7.4–15.2)	11.9 (8.0–17.4)	13.1 (8.6–20.3)
GDF-15 (ng/L)	1195.5 (827.8–1715.8)	1258.5 (907.5–1852.8)	1431.0 (1013.0–2104.8)	1722.0 (1172.5–2546.5)
CRP (mg/L)	2.0 (0.9–4.0) [1]	2.0 (1.0–4.6) [2]	2.5 (1.1–4.8) [1]	2.4 (1.1–5.2) [0]
IL-6 (ng/L)	2.1 (1.3–3.3) [0]	2.2 (1.4–3.7) [0]	2.3 (1.5–4.0) [0]	2.8 (1.7–4.9) [1]
eGFR (CKD-EPI) (mL/min)	59.3 (47.5–72.4) [0]	56.2 (45.2–67.3) [0]	55.1 (44.8–67.4) [0]	55.5 (43.9–67.5) [1]
Cystatin C (mg/L)	0.9 (0.8–1.1) [1]	1.0 (0.8–1.2) [2]	1.0 (0.8–1.2) [1]	1.0 (0.9–1.3) [0]
D-dimer (μg/L)	493.0 (326.0–809.0) [11]	523.0 (327.0–812.0) [3]	525.0 (329.0–895.5) [5]	548.0 (353.8–907.2) [7]

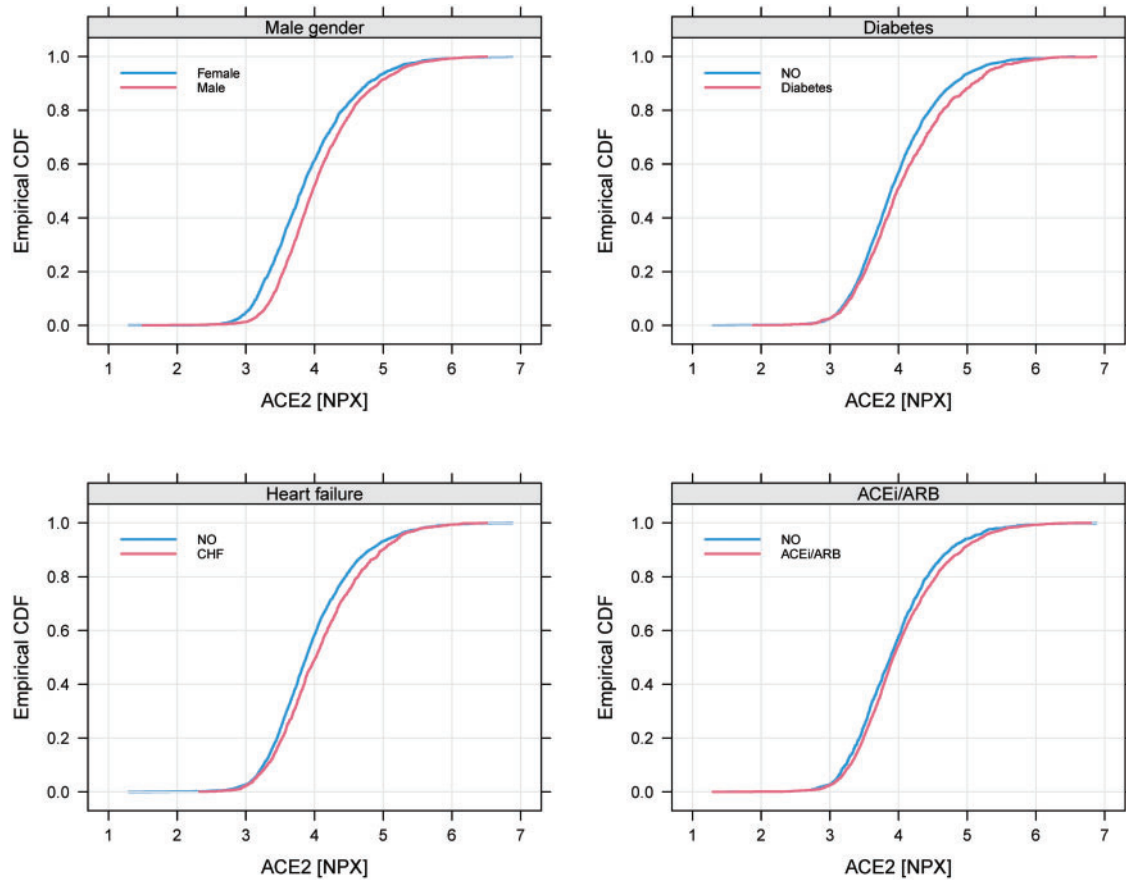
TIA, transient ischaemic attack; NT-proBNP, N-terminal probrain natriuretic peptide; hs-cTnT, high-sensitive cardiac troponin T; GDF-15, growth differentiation factor 15; CRP, C-reactive protein; IL-6, interleukin-6; eGFR, estimated glomerular filtration rate; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration. Numbers in square brackets represent missing data.

in this study are only based on within-cohort analyses and no between-cohort comparisons were performed.

The distribution of all variables in the quartile groups of sACE2 in the ARISTOTLE cohort is shown in [Table 2](#) and in the RE-LY cohort in [Supplementary material online, Table S1](#). The most striking associations were that higher quartile groups of sACE2 contained higher proportions of males, and patients with diabetes, heart failure, prior myocardial infarction, and higher levels of cardiovascular biomarkers (i.e. NT-proBNP, hs-cTnT, and GDF-15). The distributions of sACE2 in relation to the most important categorical variables are outlined in [Figure 1](#) for ARISTOTLE and in [Supplementary material online, Figure S1](#) for RE-LY. For the most important continuous variables, an estimated smooth association with the mean sACE2 is shown

in [Figure 2](#) for ARISTOTLE and in [Supplementary material online, Figure S2](#) for RE-LY. There was no statistically significant association between warfarin use within 7 days of randomization and the sACE2 levels.

The crude and adjusted effect estimates on the effects of different variables on the sACE2 level are summarized in [Figure 3A and B](#), and [Supplementary material online, Table S2](#). In unadjusted analyses and after adjustment for clinical variables and medical treatment, male sex, diabetes, congestive heart failure, prior myocardial infarction, and age were consistently associated with higher sACE2 levels in both cohorts ([Figure 3A](#); [Supplementary material online, Table S2](#)). When also adjusting for biomarker levels, only male sex and higher levels of the biomarkers GDF-15, NT-proBNP, and hs-cTnT were



**Figure 1** Cumulative distribution of soluble angiotensin-converting enzyme 2 (ACE2) for different subgroups in ARISTOTLE. CHF, Congestive heart failure; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

statistically significantly associated with higher sACE2 levels in both cohorts (Figure 3B; Supplementary material online, Table S2). However, the baseline use of an ACE inhibitor/angiotensin blocker and the level of hs-cTnT were statistically significantly associated with higher sACE2 in ARISTOTLE and directionally consistent, although not statistically significant, in the RE-LY study (Figure 3B; Supplementary material online, Table S2).

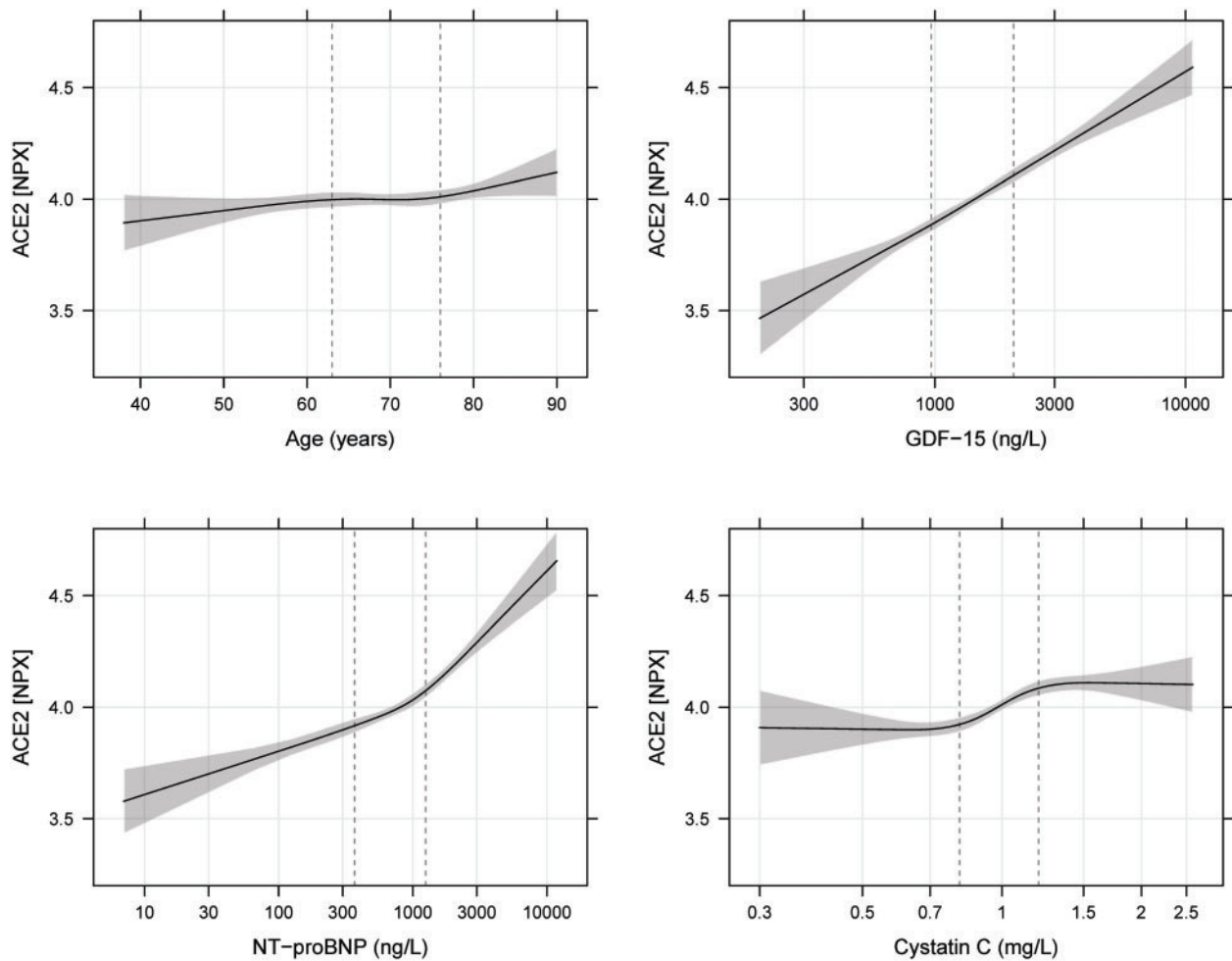
The genetic analyses showed no statistically significant associations between SNP variability and the sACE2 level when using screening across the whole genome or when focusing on the ACE2-related genetic area on the chromosome (Supplementary material online, Figure S4A and B, Table S3a and b). The results were similar whether modelled assuming inactivation of the X chromosome or not.

## Discussion

The global COVID-19 pandemic is caused by the SARS-CoV-2 virus<sup>4</sup> entering human cells using ACE2 as a receptor at the cell surface.<sup>11,12,16,17,30</sup> The present study took the opportunity to explore factors associated with circulating levels of sACE2 in two large global cohorts of elderly patients with AF and increased risk of stroke, and

thereby with an enrichment of risk factors for COVID-19 infection. The results showed that higher levels of sACE2 were associated with male sex, cardiovascular disease, diabetes, and older age, which are also the main risk factors for complications and mortality of COVID-19 infections. There were no statistically significant associations between the level of sACE2 and any of the medications for cardiovascular disease in both cohorts, although there was a trend for a higher level of sACE2 in patients using an ACE inhibitor or angiotensin receptor blocker. The sACE2 level was most strongly associated with the levels of biomarkers reflecting biological ageing, cardiovascular and renal dysfunction, and diabetes, i.e. GDF-15, NT-proBNP, and hs-cTnT. When information on these biomarkers was available, the only remaining statistically significant association between sACE2 level and clinical characteristics was the influence of male sex. An increased plasma level of sACE2 might be a reflection of a more widespread expression of ACE2 in many cells including the very richly perfused alveolar epithelium. As SARS-CoV-2 is using ACE2 as a receptor to enter cells, the increased risk of complications of COVID-19 in men and in patients with cardiovascular disease and diabetes and higher biological age might be related to higher cellular availability of ACE2. The indication that male sex and clinical or biomarker indicators of biological ageing, cardiovascular disease, and





**Figure 2** Estimated mean soluble angiotensin-converting enzyme 2 (ACE2) with 95% pointwise confidence interval for different levels of the most important continuous variables in ARISTOTLE. GDF-15, growth differentiation factor 15; NT-proBNP, N-terminal probrain natriuretic peptide.

diabetes might be associated with a specific mechanism leading to higher risk of more severe SARS-CoV-2 infection might be useful for risk stratification concerning COVID-19. Accordingly, beyond male sex and prior cardiovascular disease, higher levels of GDF-15 and NT-proBNP, which are associated both with a higher sACE2 level and a higher risk for mortality and cardiovascular complications, might contribute to better identification of risk for severe COVID-19 infection.

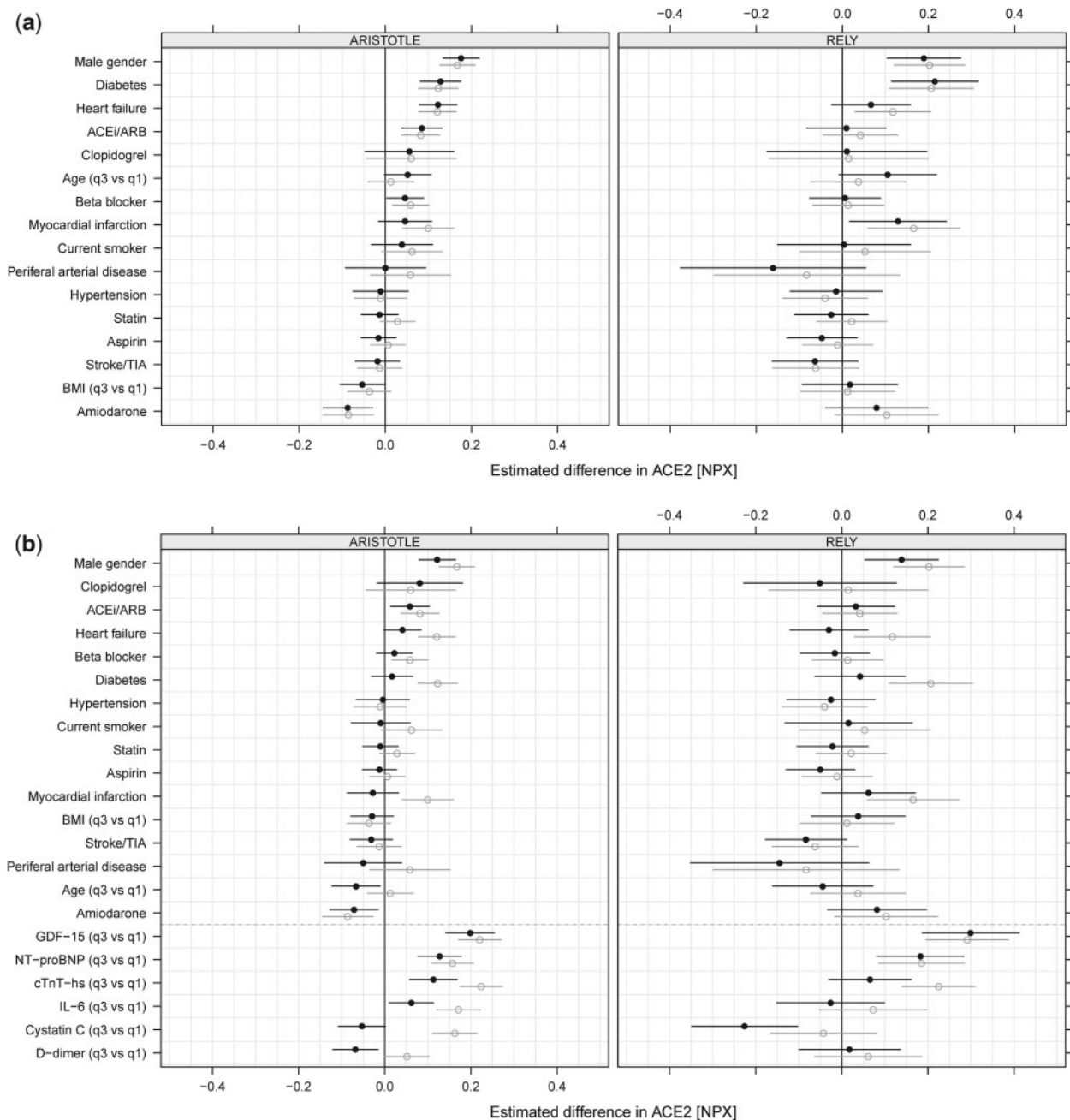
## Cellular ACE2

ACE2, a homologue of ACE, degrades angiotensin (Ang-I) and Ang-II to produce Ang-(1-9) and Ang-(1-7) which have vasodilatory properties by decreasing the levels of Ang-II and increasing the levels of Ang-(1-7).<sup>30</sup> ACE2 is expressed by a multitude of cells including endothelial, renal, myocardial, liver, and pulmonary cells. ACE2 is the internalization receptor for SARS-CoV-2 and thus functions as the main port of entry for the virus causing COVID-19.<sup>11,13,14,31</sup> Currently there are several research projects investigating whether the ACE2 pathway might be utilized as a treatment target for SARS-CoV-2 infection.

Hypothetically, infusion of an abundance of ACE2 might compete with the binding of SARS-CoV-2 to cell membranes, while TMPRSS2 inhibitors might prevent the activation of the spike protein and thereby impair the binding to the ACE2 receptor.<sup>12,32</sup>

## Soluble ACE2

sACE2 protein, containing the catalytic site of the enzyme, is shed into the circulation after the cleavage of the membrane-bound molecule by a disintegrin and metalloprotease 17 (ADAM17) or tumour necrosis factor- $\alpha$ -converting enzyme (TACE).<sup>11,16,17,30</sup> There is limited knowledge of the regulation of ACE2 expression in different cells and also of the association between circulating sACE2 levels and the cellular levels of ACE2 in humans. So far, the few published studies on circulating sACE2 concern measurements of sACE2 activity, which in a few small studies has been shown to be higher in older individuals, males, and those with cardiovascular disease.<sup>30,33,34</sup> In another study in patients with chronic kidney disease, the ACE2 activity was also higher in males and was associated with atherosclerotic lesions.<sup>35</sup> In a study of patients with chronic coronary artery disease,



**Figure 3** (A) Estimated difference of soluble angiotensin-converting enzyme 2 (sACE2) based on the models with clinical variables and treatments for ARISTOTLE (left panel) and RE-LY (right panel). (B) Estimated difference of sACE2 based on the models with clinical variables, treatments, and biomarkers for ARISTOTLE (left panel) and RE-LY (right panel). Unadjusted results (grey, open circles) and adjusted results (black, filled circles). Variables are sorted after estimated effect size in ARISTOTLE. Note that effect sizes for continuous variables are, for a comparison between the third and the first quartile in both studies, estimated using only the ARISTOTLE data. ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; TIA, transient ischaemic attack; BMI, body mass index. NT-proBNP, N-terminal probrain natriuretic peptide; cTnT-hs, high-sensitive cardiac troponin T; GDF-15, growth differentiation factor 15.

there was higher sACE2 activity in males and an association with cardiovascular events during 10 years follow-up.<sup>36</sup> The sACE2 activity has also been found to be elevated in diabetes with vascular complications.<sup>37</sup> Recently it was reported from two cohorts of 2017 and

1698 patients with heart failure that male sex was the strongest clinical predictor of the sACE2 level measured by the same method as in the current study.<sup>38</sup> Therefore, our results concerning the sACE2 protein level are in accordance with previous findings of an

association between ACE2 and male sex and the occurrence of cardiovascular disease and diabetes.

## Clinical risk factors

During the last months, >2.1 million people have been diagnosed with COVID-19, of which >146 088 have died (WHO Covid-19 Situation Report-89, 18 April 2020 at 10.00 h CET). Based on a few scientific reports and a wealth of information on the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) websites, it is well established that both occurrence and severity of COVID-19 increase with age, male sex, and comorbidities such as cardiovascular diseases and diabetes.<sup>1,8,9</sup> Based on the current demonstration of associations between sACE2 and these risk factors, it might be hypothesized that males and elderly persons with cardiovascular disease or diabetes have an increased expression of ACE2 leading to binding of more SARS-CoV-2 virus particles and, accordingly, a more severe infection. The increased rate of complications and poor outcomes of COVID-19 in these groups may also be driven by unopposed renin–angiotensin–aldosterone system (RAAS) activation due to SARS-CoV2-mediated down-regulation of the previously up-regulated ACE2. Future studies measuring sACE2 pre- and post-infection are therefore warranted to investigate if sACE2 might have a role as a predictor of outcomes in patients with COVID-19 infections.

## ACE inhibitors

Concerns have been raised about the influence of ACE inhibitors and angiotensin receptor blockers on the expression of ACE2. In our study, there was an association between renin–angiotensin system (RAS) inhibitor treatment and the sACE2 level in the larger cohort and a trend in the same direction in the smaller cohort. Therefore, these results support the need for further investigation of the effects of RAS inhibitors on ACE2 expression and COVID-19 infection.<sup>13,39</sup>

## Cardiovascular biomarkers

The present data showed that the cardiovascular biomarkers GDF-15, NT-proBNP, and hs-cTnT had the strongest associations with the sACE2 level and stronger associations than any of the clinical characteristics. Also two previous small studies of ACE2 activity reported statistically significant associations between sACE2 activity, heart failure, AF, myocardial function, and a higher level of BNP.<sup>33,34</sup> Several previous reports have documented that these biomarkers are stronger indicators of cardiovascular dysfunction and disease than clinical risk factors. Both in elderly healthy individuals<sup>40,41</sup> and in patients with previous cardiovascular disease,<sup>18–20,42–44</sup> these biomarkers are strong risk indicators for cardiovascular outcomes such as myocardial infarction, congestive heart failure, stroke, and cardiovascular death. NT-proBNP and hs-cTnT are specific indicators of myocardial dysfunction and necrosis, and also increase with age. GDF-15 is an unspecific indicator of cellular stress, inflammation, and biological ageing, which, besides being elevated with cardiovascular dysfunction, is also increased with diabetes mellitus, renal dysfunction, and increased chronological age. These biomarkers therefore seem to reflect biological ageing, as well as cardiovascular dysfunction and diabetes, which might be the reason why chronological age is not statistically significantly associated with sACE2 after multivariable adjustment. The close association between biomarkers and the sACE2

level suggests that biological ageing and cardiovascular disease and dysfunction might lead to increased ACE2 expression and a potentially higher risk for SARS-CoV-2 binding and more severe COVID-19 infection.

## GWAS

In this study we were unable to show any genetic regulation of the sACE2 level either in women or in men. In a previous small study of 213 subjects,<sup>45</sup> there was, in the 111 women, but not in the men, an association between reduced level of sACE2 and rare alleles of ACE2 rs2074192 and rs2106809, which, however, was not verified in the present analyses. Neither were there any differences between the sACE2 level in different ethnic or geographic groups in these analyses (data not shown). Therefore, these data do not support that different expression of ACE2 might explain any differences in COVID-19 infection between different geographic areas or different ethnic groups.

## Limitations

The present study has several limitations. The main limitation is that currently there is no information about the association between ACE2 bound to cell membranes and circulating sACE2 levels. There is also no information on the expression of ACE2 in different cells and organs and the sACE2 level. In addition, it is unknown whether the variability of cellular ACE2 and/or sACE2 has any influence on the risk for COVID-19 infection or its severity. The investigated populations of patients with AF at increased risk of stroke contain mainly high-risk elderly patients with a median age of 70–72 years and 79–88% with hypertension, making it not feasible to evaluate the associations between sACE2 and hypertension, AF, and the full range of age. These data were generated in cohorts of mainly white European ancestry and therefore these results might be generalizable only to similar populations.

## Conclusions

In two large international cohorts of elderly patients with AF, the level of circulating sACE2 was associated with male sex, cardiovascular disease, diabetes, and age. The sACE2 level was most strongly associated with the levels of the biomarkers GDF-15 and NT-proBNP, reflecting biological ageing and cardiovascular and renal dysfunction, and diabetes. As SARS-CoV-2 is using ACE2 as a receptor to enter cells, the increased risk of complications of COVID-19 in men, those of higher biological age, and those with cardiovascular disease and diabetes might be related to a higher sACE2 level reflecting a higher cellular availability of ACE2.

## Implications

Measurements of the routinely available biomarkers GDF-15 and NT-proBNP, which are associated both with higher sACE2 level and a higher risk for mortality and cardiovascular disease, might be useful in better identifying patients who are at high risk for severe COVID-19 infection.



## Supplementary material

Supplementary material is available online at *European Heart Journal*.

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