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EDITORIAL

New data on soluble ACE2 in patients with atrial fibrillation reveal potential value for treatment of patients with COVID-19 and cardiovascular disease

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This editorial refers to 'Angiotensin-converting enzyme 2 (ACE2) levels in relation to risk factors for COVID-19 in two large cohorts of patients with atrial fibrillation', by L. Wallentin *et al.*, doi:10.1093/eurheartj/ehaa697.

The coronavirus disease 2019 (COVID-19) pandemic is still uncurbed partially because its pathogenesis is not well understood. Interestingly, cardiovascular complications are rapidly emerging as susceptibility factors and a key threat in COVID-19 in addition to respiratory disease. Indeed, in a recent autopsy report of 80 patients who died of COVID-19 in Germany, most had diseases of the cardiovascular system (85%), followed by diseases of the lungs (55%), central nervous system (35%), kidneys (34%), and diabetes mellitus (21%).[1] Furthermore, COVID-19 pneumonia was found in 83% and deep vein thrombosis in 40% of the deceased cases.[1] Earlier studies show that COVID-19 risk factors include older age, male sex, hypertension, diabetes, coronary heart disease, and obesity; and the common outcomes include organ damage (lungs, kidneys, and heart), lymphocytopenia, thrombocytopenia, and death.[2]

Angiotensin-converting enzyme type 2 (ACE2) is a protein at the cross-talk between cardiovascular diseases and COVID-19 because (i) ACE2 is the functional cellular receptor for the severe acute respiratory syndrome coronavirus (SARS-CoV) and SARS-CoV-2 which causes COVID-19; and (ii) ACE2 is a pivotal cardioprotective protein in the renin–angiotensin–aldosterone system (RAAS) where it converts angiotensin II to angiotensin-(1-7) in order to reduce elevated blood pressure, hypertrophy, and fibrosis. The ACE2 protein is anchored on cell membrane surfaces in tissues such as the lungs, heart, kidneys, testis, and endothelium, but also circulates in plasma as a soluble form (reviewed in Gheblawi *et al.*[3] and Sama *et al.*[4]). Soluble ACE2 (sACE2) is shed from membrane-bound ACE2, a process modulated by ADAM17 (a disintegrin and metalloproteinase 17).[5] Importantly, membrane-bound ACE2 is lost by degradation post coronavirus infection, and by enhanced shedding into plasma due to angiotensin II, diabetes, and SARS-coronavirus activation of ADAM17.[3,6] This common loss of membrane-bound ACE2 similarly culminates in diminishment of ACE2-related tissue protection in cardiovascular disease and COVID-19 (*Take home figure*). However, the balance between ACE2 seen in plasma and in ACE2-expressing tissues is unknown, and this obfuscates interpretation of levels of sACE2. Moreover, the paucity of matched plasma and tissue ACE2 data persists because it is prohibitively invasive to obtain tissue samples from all ACE2-expressing organs in patients. Therefore, despite the uncertainties about the relationship between soluble and membrane-bound ACE2, studies on sACE2 from plasma remain of great interest.

In this issue of the *European Heart Journal*, Wallentin *et al.* have explored the associations between sACE2, clinical factors, and genetic variability in two international cohorts of elderly patients with atrial fibrillation.[7] They used pre-COVID-19 plasma samples from a subset of ARISTOTLE (n = 3999) and RE-LY (n = 1088). Plasma sACE2 was measured using the Olink Proteomics® Multiplex CVD II96 × 96 panel. Additional cardiovascular biomarkers such as high-sensitive cardiac troponin T (hs-cTnT), N-terminal pro brain natriuretic peptide (NT-proBNP), and growth differentiation factor 15 (GDF-15) were measured using immunoassays. Results from both cohorts were largely similar, with hypertension, diabetes, and chronic heart failure being predominant comorbidities. Importantly, male sex was

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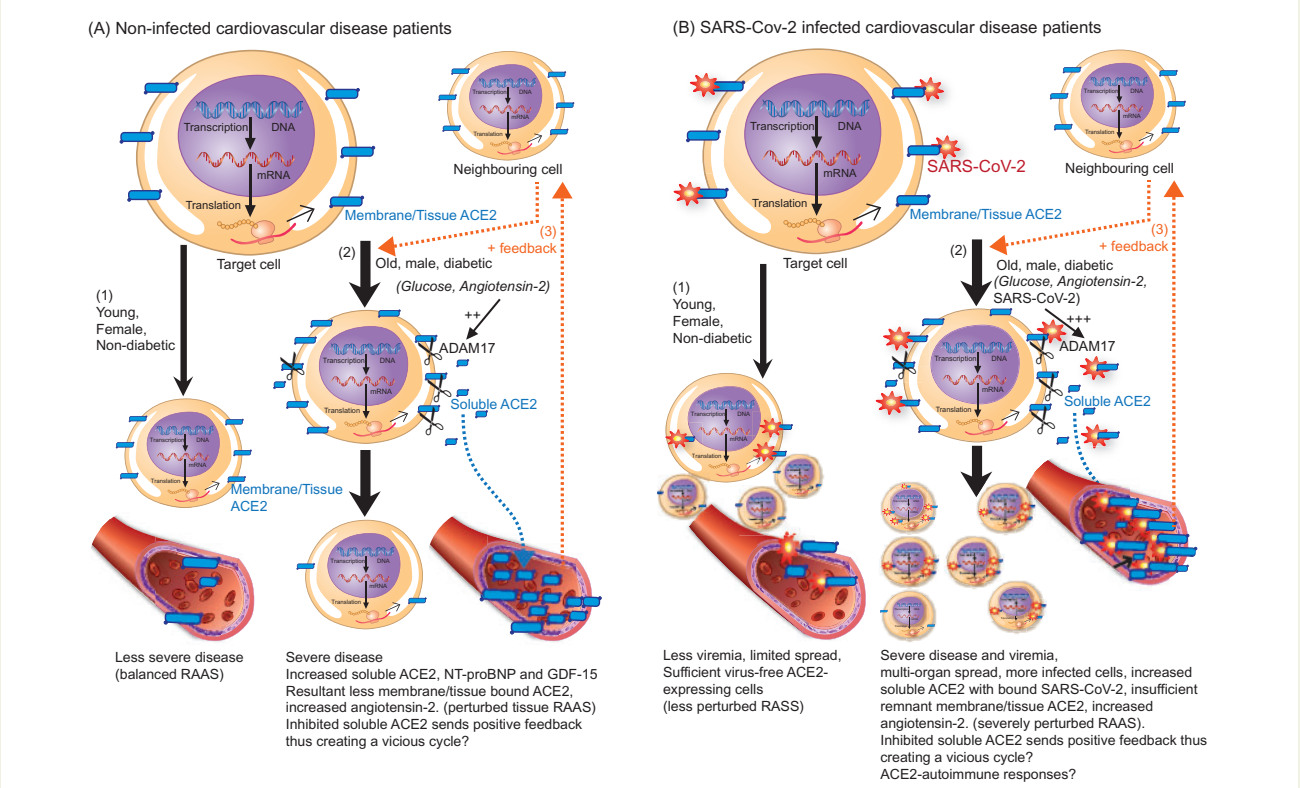

Take home figure Hypothetical representation of the regulation of ACE2 in cardiovascular disease with and without SARS-CoV-2 infection. The COVID-19 pandemic accentuates the failure to adequately inhibit the RAAS-associated increase in morbidity and mortality of patients with cardiovascular diseases. We therefore need to learn more about the role of ACE2 in relation to an activated RAAS and risk factors causing higher/lower ACE2 activity. (*A*) In non-infected patients, male sex and diabetes are associated with elevated levels of soluble ACE2 (sACE2) which can be shed by ADAM17 from membrane-bound ACE2 in tissues into the circulation. The loss of membrane-bound ACE2 elevates angiotensin II, and thus perturbs tissue RAAS. Higher concentrations of sACE2 are associated with greater disease severity (diabetes, congestive heart failure) and can be identified by elevated concentrations of circulating biomarkers, such as NT-proBNP, hs-cTnT, GDF-15, and D-dimer (Wallentin *et al.*[7]). The enzymatic activity of ACE2 is highly pH dependent (almost inactive at pH 5.0).[14] In acidic blood (e.g. in diabetes), lower activity of sACE2 will increase angiotensin II concentrations. An elevated angiotensin II in turn could inhibit ACE2 activity[13] and might forge a positive feedback loop for more ACE2 expression. (B) Upon eventual SARS coronavirus encounter, cells that still have ACE2 as receptors (including neighbouring cells that were induced to express more ACE2 by the feedback loop) will internalize and replicate the virus. Cells expressing viral particles on their surfaces can also bind neighbouring ACE2-expressing cells to form syncytia, further increasing viral yield and spread. Meanwhile some sACE2, virus, and virus-bound ACE2 will be shed into the circulation. Consequently, membrane-/tissue-bound ACE2 is further depleted, leading to a vicious cycle of worsening RAAS perturbation and increased viraemia and induction of ACE2 expression. Increasing levels of virus-bound soluble ACE2 (which is not an antibody–antigen complex) in the circulation might lead to thrombotic vascular occlusion, autoimmune inflammation, coagulopathy, and multiorgan ischaemia. These outcomes have been observed in COVID-19 patients. If the positive feedback loop were not plausible, virus re-infection and replication would be limited.

the strongest independent predictor of sACE2 levels, thus corroborating previous reports[4]. Furthermore, GDF-15, NT-proBNP, hs-cTnT, and D-dimer, which are indicators of cardiovascular disease, diabetes, biological ageing, coagulopathy, and mortality, were associated with higher sACE2 levels. Using DNA from whole blood samples, they further investigated genetic variability to explain plasma ACE2 levels by performing genome-wide association studies (GWAS) in a smaller portion of patients (ARISTOTLE subset n = 1583/3999 and RE-LY subset n = 289/1088). No significant genetic association was found. This was partly due to the limited data, which was an acknowledged study limitation. Indeed, in a recent GWAS of sex-specific genotype effects on plasma ACE2 concentrations, we identified three loci of genome-wide significance in men (n = 2420), but none in women (n = 1022).[8] Interestingly, the strongest association was with an X chromosome locus around the *ACE2* gene (rs12558179). The second association was on chromosome 12 around the *HNF1α* gene (rs71076692 and rs7139079), which encodes a transcription factor that induces ACE2 expression in pancreatic islets.[9] The third association was on chromosome 21 around the *ERG* gene (rs2186346) which is involved in vascular development and remodelling. Notably, *ERG* is often involved in fusion gene products, such as *TMPRSS2-ERG* in prostate cancer. The TMPRSS2 (transmembrane protease serine 2) protein modulates cleavage and entry of SARS-CoV-2 into cells (reviewed in Gheblawi *et al.*[3]). These associations need to be further explored.

The work of Wallentin *et al.* is important and timely because it contributes to the growing body of research aimed at deciphering ACE2 pathophysiology and possible implications in COVID care, as

summarized in *Take home figure*. First, these data confirm previous findings showing that in patients with heart failure (but without SARS-coronavirus infection), elevated sACE2 concentrations (ng/mL) was associated with greater severity of myocardial dysfunction and was an independent predictor of adverse clinical events.[10] In a small pilot trial in patients with acute respiratory distress syndrome, a recombinant human ACE2 (rhACE2) decreased angiotensin II while increasing angiotensin-(1-7) levels.[11] Interestingly, a recent report in patients with heart failure show that an elevated angiotensin-(1-7)/angiotensin II ratio is an independent predictor of beneficial outcomes (higher survival rate and decreased duration of hospitalization).[12] Notably, the enzymatic activity of ACE2 is inhibited by high substrate concentrations,[13] and by an acidic pH[14] such as might be the case in the blood of diabetic patients. Therefore, angiotensin-(1-7) supplementation to increase the angiotensin-(1-7)/angiotensin II ratio might be a useful strategy to bypass sACE2 anomalies/insufficiency to circumvent a perturbed RAAS.

Secondly, data on sACE2 in COVID-19 patients is lacking and the clinical trial (NCT04287686) that was aimed at assessing rhACE2 in COVID-19 patients has been withdrawn without further details. However, speculations from ACE2 diabetes biology might help, since diabetes is a common COVID-19 risk factor[1,2] and, as confirmed by Wallentin *et al*., is also associated with elevated plasma ACE2 concentrations. Furthermore, an ACE2-deficient pancreas can exacerbate or cause diabetes when insulin production is compromised, as previously observed in SARS patients.[15] When diabetes, elevated angiotensin II, and SARS-coronavirus infection activate ADAM17 to enhance shedding of membrane-bound ACE2, this would exacerbate tissue RAAS perturbation, leading to fibrosis, and also increase sACE2-bound virus in the circulation (*Take home figure B*). Circulating SARS-CoV2–ACE2 complex is not an antibody–antigen complex, and thus is not earmarked for opsonization and classical immune clearance. Rather, it might lead to vascular occlusions and subsequent organ ischaemia. Moreover, an immune reaction targeting the whole SARS-CoV2–ACE2 complex might initiate autoimmune responses against the ACE2 portion. Autoimmune inflammation[16] and deep vein thrombosis[1] are common in COVID-19.

Although supplementation with rhACE2 in non-infected patients with cardiovascular diseases and an activated RAAS might be beneficial,[10] this is probably not the case in COVID-19 patients. Direct supplementation with the ACE2 end-product, angiotensin-(1-7) might help mitigate clinical complications in COVID-19 patients and reduce the need for ACE2 expression; thus curbing (re)-infection and autoimmune risks. Furthermore, adjusting blood pH to the optimal 6.5 and low basic range[13] might unblock sACE2 activity. Alternatively, a depletion of membrane-bound ACE2 might limit COVID-19 severity since fewer viruses will enter ACE2-deficient cells. These hypotheses need to be further investigated and might guide and inspire ongoing and future research.

Meanwhile, the COVID-19 pandemic is ongoing. While awaiting results from randomized clinical trials, and any on the stoichiometric balance between tissue and plasma ACE2, the work by Wallentin *et al*. is important as it adds scientific and clinical data on ACE2 biology from individuals that are highly susceptible to COVID-19. Monitoring the plasma levels of ACE2, the angiotensin-(1-7)/angiotensin II ratio, pH, the ACE2/ACE ratio, GDF-15, and NT-proBNP before, during, and post SARS-CoV-2 infection might contribute to better identification of risk for severe disease and might eventually guide treatment modalities to cure COVID-19 and other ACE2-related diseases.

Conflict of interest: none declared.

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