Plasma Angiotensin Peptide Profiling and ACE (Angiotensin-Converting Enzyme)-2 Activity in COVID-19 Patients Treated With Pharmacological Blockers of the Renin-Angiotensin System

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Pharmacological blockade of the renin-angiotensin system (RAS) with ACE (angiotensin-converting enzyme) inhibitors or angiotensin type 1 receptor blockers (ARB) reduces morbidity and mortality in various cardiovascular diseases. One of the key RAS-modulating enzymes, ACE2, has recently gained increasing attention because it converts not only angiotensin (Ang) II to the alternative RAS metabolite Ang-(1–7) but also functions as the cellular entry receptor for SARS-CoV-2.[1] At the beginning of the SARS-CoV-2 pandemic, some investigators suggested that because ACE inhibitor or ARB may lead to upregulation of ACE2 expression/activity, use of these agents in coronavirus disease 2019 (COVID-19) patients might be associated with worsened outcomes.[1] Meanwhile, several observational studies have shown that neither the risk of COVID-19 nor its severity is negatively affected by ACE inhibitor or ARB.[2,3] However, it remains unclear how RAS activity, particularly ACE2, is regulated in COVID-19 and how this is altered by ACE inhibitor/ARB therapy. In this study, we analyzed distinct RAS components in plasma from COVID-19 patients±ACE inhibitor/ARB therapy using LC-MS/MS.

The study was approved by the Charité-Universitätsmedizin, Berlin, Germany, Institutional Ethics Committee (EA2/204/19, Amendment 1) and registered in the German Registry for Clinical Studies (DRKS00019207). Surplus plasma samples were collected at the time of admission to the emergency room from 6 different patient groups (total, n=58 [women, 21]): SARS-CoV-2 negative control group (CTRL, n=9 [4]), SARS-CoV-2 negative with ACE inhibitor (CTRL-ACE inhibitor, n=10 [2]), SARS-CoV-2 negative with ARBs (CTRL-ARB, n=8 [5]), COVID-19 without ACE inhibitor/ARB (COVID, n=12 [5]), COVID-19 with ACE inhibitor (COVID-ACE inhibitor, n=10 [2]), and COVID-19 with ARBs (COVID-ARB, n=9 [3]). Equilibrium levels of Ang-peptides (Ang I, Ang II, Ang-[1–7], and Ang-[1–5]) were measured using LC-MS/MS technology (Attoquant Diagnostics).[4] Ang-based markers for ACE (Ang II/Ang I) and plasma renin activity (Ang I+Ang II) were calculated from Ang-peptide levels. ACE2 activity was assayed by a classical kinetic approach applying its natural substrate (ex vivo spiked Ang II) and measuring the turnover to Ang-(1–7)±ACE2 inhibitor MLN-4760. The inhibitor-sensitive ACE2-specific turnover was converted to an ACE2 concentration using a calibration curve of recombinant human ACE2. Ang-peptide concentrations/ratios, ACE2 activity, and age between groups were compared using the Kruskal-Wallis test. In case of a significant result, the Dunn-Test for pairwise comparisons using Bonferroni correction was applied. A P of <0.05 was considered statistically significant, although results have to be considered exploratory.

Patient Characteristics

Age (years, mean±SD): CTRL, 44.8±19.7; CTRL-ACE inhibitor, 63.6±17.8; CTRL-ARB, 73.1±11.4 (P=0.02 versus CTRL); COVID, 50±15.1; COVID-ACE inhibitor, 61.4±20.9; COVID-ARB, 74.2±10.1 (P=0.02 versus COVID); COVID severity (n/group), as defined previously,[3] severe (ICU admission, mechanical ventilation, and death): COVID (2), COVID-ACE inhibitor (1), COVID-ARB (1); acute renal failure ([n/group] CTRL-ARB [2], COVID [1], COVID-ARB [1]); diuretic use (n/group): CTRL (0), CTRL-ACE inhibitor (4), CTRL-ARB (6), COVID (0), COVID-ACE inhibitor (1), and COVID-ARB (3). Coexisting conditions are outlined in the Figure (A).

Ang-peptide equilibrium concentrations did not significantly differ between the CTRL and COVID groups without ACE inhibitor/ARB treatment (Figure [B], left). More importantly, Ang I+II, Ang II/Ang I, and ACE2 activity were not significantly different between both groups (Figure [C]). These data suggest that COVID-19 patients are not those with increased RAS activity levels and that particularly COVID-19–induced alternative RAS activation, potentially mediated through circulating ACE2, is not a typical feature in our patient cohort.

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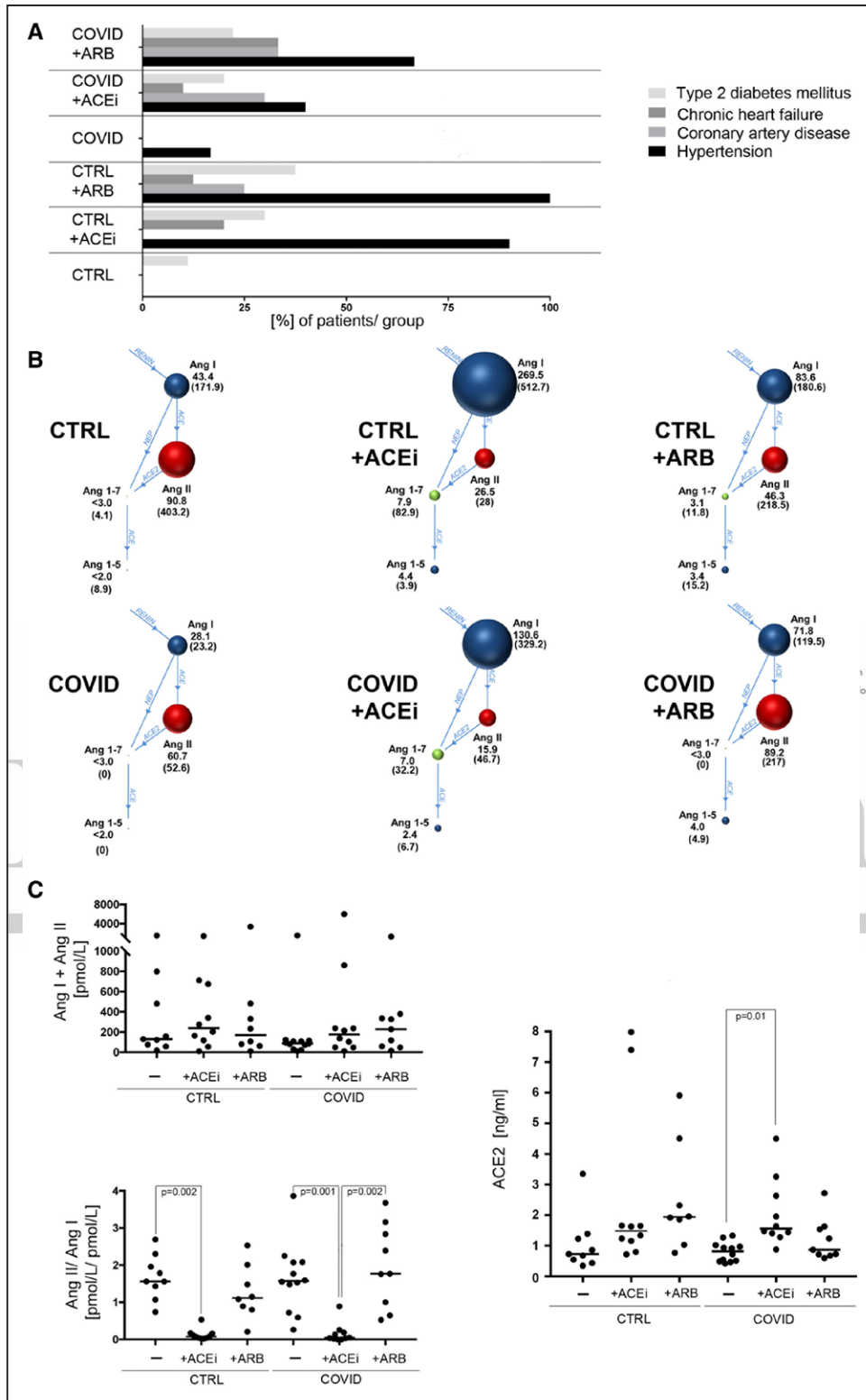
Figure. Patient characteristics, Ang (angiotensin) peptide profiles, and ACE (angiotensin-converting enzyme)-2 levels. **A**, The presence of cardiovascular disease (hypertension, coronary artery disease, and chronic heart failure) and type 2 diabetes mellitus depicted as percentage of patients in each group. **B**, Plasma Ang-peptide concentrations and renin-angiotensin system (RAS) enzymatic cascade are depicted as RAS Fingerprints. The concentration of indicated Ang metabolites is reflected by the size of the corresponding sphere. Blue arrows indicate enzymes that are known to carry out metabolic conversions between connected Ang metabolites. Numbers represent median concentrations (pmol/L) and interquartile ranges in parentheses. **C**, Ang-based markers for plasma renin activity: Ang I+Ang II and ACE: Ang II/Ang I were calculated from Ang-peptide levels. ACE2 activity was measured as described above. Data are shown as dot plots and median. Significant *P* values within each group (CTRL and coronavirus disease 2019 [COVID]) are indicated. CTRL: SARS-CoV-2 negative CTRL group without ACE inhibitor/angiotensin type 1 receptor blocker (ARB) therapy; CTRL+ACE inhibitor: SARS-CoV-2 negative CTRL group with ACE inhibitor therapy; CTRL+ARB: SARS-CoV-2 negative CTRL group with ARB therapy; COVID: COVID-19 patients without ACE inhibitor/ARB; COVID+ACE inhibitor: COVID-19 patients with ACE inhibitor therapy; COVID+ARB: COVID-19 patients with ARB therapy.

Comparison of all groups, including ACE inhibitor/ARB treatment groups, revealed no significant differences of Ang I+II levels between the groups (Figure [C], upper left). Ang I+II is a reliable marker for plasma renin activity and did not change significantly, despite the use of ACE inhibitor/ARB, while median values were clearly increased in patients on ACE inhibitor/ARB. This is consistent with previous observations demonstrating a broad spectrum of intensity in compensatory renin secretion in patients treated with ACE inhibitor or ARB.[4] As expected, patients in the CTRL-ACE inhibitor and COVID-ACE inhibitor group showed increased Ang I and markedly suppressed Ang II levels (Figure [B]), resulting in a significant reduction of the Ang II/Ang I ratio (Figure [C], lower left). Ang-(1–5) levels did not significantly differ between groups, whereas Ang-(1–7) was significantly increased in the COVID-ACE inhibitor group versus COVID without ACE inhibitor/ARB ($P=0.01$) and versus COVID-ARB ($P=0.045$). ACE2 activity was significantly higher in COVID-19 patients treated with ACE inhibitor compared with COVID-19 patients without ACE inhibitor/ARB (Figure [C], right). ACE2 activity was also increased in the CTRL-ACE inhibitor and CTRL-ARB group but did not reach statistical significance (Figure [C], right). ARB treatment in COVID-19 did not significantly affect ACE2 activity (Figure [C], right).

The main findings of this study are as follows: (1) COVID-19 patients are not characterized by major changes in RAS activity in plasma including ACE2 activity, (2) ACE inhibitor therapy significantly suppressed Ang II/Ang I ratios, the Ang-based marker for ACE, in COVID-19 and in non–COVID-19 patients, and (3) plasma ACE2 activity is increased in COVID-19 patients treated with ACE inhibitor. These data are consistent with previously published results in SARS-CoV-2–negative patients treated with ACE inhibitor or ARB demonstrating an Ang II/Ang I suppression and a more profound increase of Ang-(1–7) under ACE inhibitor compared with ARBs.[4] The data published so far on plasma ACE2 activity and Ang-(1–7) levels in patients without COVID treated with ACE inhibitor or ARBs are controversial.[1] Some studies showed an increase in circulating ACE2 activity and Ang-(1–7) levels that cannot be proven by other studies.[1] In addition, increased ACE2 activity has been identified in multiple cardiovascular diseases such as hypertension, CAD, and CHF, which are usually treated with ACE inhibitor.[1] Whether the ACE inhibitor treatment in our study plays a role in ACE2 upregulation or whether these changes are mediated by the increased presence of cardiovascular disease in this group requires further investigation. Furthermore, the clinical significance of the elevated ACE2 activity in COVID-19 patients treated with ACE inhibitor is currently not completely understood. Whether plasma ACE2 level may be a reliable marker of the full-length membrane bound form[1] and whether ACE2 serves as a marker for disease severity or endothelial regeneration in the lung[5] need to be clarified in future studies. Some of the major limitations of this study include small sample sizes, lack of a power analysis, lack of any data on blood pressure when the plasma samples were obtained, and lack of any data on duration of illness. Finally, it should be emphasized that the majority of the study patients were not experiencing severe COVID-19. However, we provide for the first time a snapshot of distinct systemic RAS components in COVID-19 patients under ACE inhibitor/ARB therapy that helps to understand the clinical data on a molecular pharmacological level.

Disclosures
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