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Early View

Research letter

Plasma ACE2 activity is persistently elevated following SARS-CoV-2 infection: implications for COVID-19 pathogenesis and consequences

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Plasma ACE2 activity is persistently elevated following SARS-CoV-2 infection: implications for COVID-19 pathogenesis and consequences

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COVID-19 causes persistent endothelial inflammation, lung, cardiovascular, kidney and neurological complications as well as thromboembolic phenomena of unclear pathogenesis [1]. SARS-CoV-2 utilises the catalytic site of full-length membrane-bound angiotensin converting enzyme 2 (ACE2) for host cell entry [2], which is thought to downregulate membrane-bound ACE2, and thus contribute to ongoing inflammation due to loss of a degradative pathway for angiotensin II. In healthy individuals, ACE2 exists primarily in its membrane-bound form with very low levels of the catalytically active ectodomain of ACE2 present in the circulation [3]. However, in patients with cardiovascular disease, there is increased "shedding" of ACE2, and higher circulating levels are associated with downregulation of membrane-bound ACE2 [4].

In this prospective observational study in recovered COVID-19 patients, we hypothesised that SARS-CoV-2 infection would be associated with shedding of ACE2 from cell membranes leading to increased plasma ACE2 activity levels.

We measured plasma ACE2 catalytic activity using a validated, sensitive quenched fluorescent substrate-based in-house assay as previously described [3-5] in a cohort of Australians aged $\geq$18 years (n=66) who had recovered from mild, moderate or severe SARS-CoV-2 infection (positive result by PCR testing) and age and gender matched uninfected controls (n=70). Of the 66 recovered SARS-CoV-2 patients, 23 agreed to provide follow-up samples. The severity of COVID-19 infection was defined according to National Institutes of Health COVID-19 treatment guidelines [6] as mild (predominantly upper respiratory tract infection), moderate (lower respiratory tract infection) or severe (hospitalisation). The immune responses in a subset of the overall cohort in this paper have been previously reported [7], and for the purpose of this study, we also obtained information on medications and comorbid conditions including hypertension (diagnosis of hypertension and/or anti-hypertensive treatment), hyperlipidaemia (treatment with lipid lowering agents), obesity (body mass index $\geq$30 kg/m^2), diabetes and cardiovascular disease. The study was approved by the University of Melbourne Human Research Ethics Committee (#2056689); all participants provided written informed consent in accordance with the Declaration of Helsinki.

Data were analysed with SPSS version 26.0 (IBM SPSS Statistics, IBM Corp©, USA). Plasma ACE2 activity was compared using the Mann-Whitney test or Kruskal–Wallis test and significance adjusted by Bonferroni correction for multiple tests. Fisher's Exact test was used to compare categorical variables. Serial ACE2 activity levels were analysed using the Friedman test for repeated measures; after post hoc analysis with Wilcoxon signed-rank tests with a Bonferroni correction applied. Two-tailed P-values <0.05 were considered significant.

The controls and SARS-CoV-2 recovered subjects were matched for age (54 ± 11 vs. 53 ± 14 years, p=0.47) and gender (37 (53%) vs. 39 (59%) male, p=0.49) (mean ± SD). With regard to risk factors and/or comorbidities, hypertension was present in 7% (n = 9) of all subjects, hyperlipidaemia in 8% (n = 11), obesity in 2% (n = 3), diabetes in 1% (n = 1) and cardiovascular disease in 2% (n = 3), with no difference between controls and SARS-CoV-2 recovered subjects (all, P >0.05). There was no difference in the use of antihypertensive (n = 7), lipid lowering (n = 11) or antiplatelet therapy (n =4) between controls and SARS-CoV-2 recovered subjects (all, P >0.05).

At a median of 35 days post-infection [interquartile range 30-38 days], plasma ACE2 activity was significantly higher in recovered SARS-CoV-2 subjects compared to controls (5.8 [2-

11.3] vs. 0.06 [0.02-2.2] pmol/min/ml, p<0.0001, Figure 1A). Plasma ACE2 activity differed according to disease severity (p=0.033) with severe COVID-19 associated with higher ACE2 activity compared to mild disease (11.2 [8.3-23.2] vs. 5.4 [1.8-9.0] pmol/min/ml (Figure 1B, p=0.027). Men (n=39) who were SARS-CoV-2 positive had higher median plasma ACE2 levels compared to women (n=27) (9.2 [5.8-15.3] vs. 2.1 [0.2-5.1] pmol/min/ml p<0.0001). The observation that plasma ACE2 is higher in men compared to women is consistent with our previous findings in non-COVID-19 patients with coronary artery disease [5], and in the general population [8].

We next analysed whether elevated plasma ACE2 activity persisted following SARS-CoV-2 infection in 23 subjects who provided further blood samples at 63 [56-65] and 114 [111-125] days post-infection. This subset was representative of the overall cohort for age (52 ± 15 years), gender (14 (61%) male) and disease severity (mild 15 (65%), moderate 5 (22%), severe 3 (13%). Plasma ACE2 activity remained persistently elevated in almost all subjects (Figure 1C), with no significant differences between timepoints in post-hoc comparisons (p>0.05). There was no change in plasma ACE2 activity in control subjects with serial samples (n=8, timepoint 1: 0.8 [0.02-3.0] vs. timepoint 2: 1.4 [0.02- 5.9] pmol/min/ml, p=0.31).

This is the first description that plasma ACE2 activity is elevated in patients after COVID-19 infection compared to uninfected age and gender matched controls with similar comorbidities, and the first with long-term data to indicate that plasma ACE2 activity remains elevated out to a median of 114 days post-infection, the last time point sampled. It is already known that males have higher plasma ACE2 activity than females [5,8], but this is the first report that plasma ACE2 activity levels are increased in those with more severe COVID-19 infection. Although the pathophysiologic cause and consequences of elevated plasma ACE2 activity in the setting of COVID-19 are unknown, we reported that increased plasma ACE2 was an independent risk factor for major adverse cardiac events in patients with aortic stenosis [4] and coronary artery disease [5]. Others have recently shown that higher plasma ACE2 concentration was associated with increased risk of major cardiovascular events in a large global population-based study [8].

In conclusion, we suggest that binding of SARS-CoV-2 to membrane ACE2 has at least 2 effects. First, it downregulates membrane ACE2 causing a dysregulated local renin angiotensin system (RAS) that favours inflammation and ongoing tissue damage secondary to excess angiotensin II [9]. Second, the dysregulated local RAS is associated with prolonged shedding of the catalytically active site of ACE2 into the circulation.

In addition to the early multisystem effects of COVID-19, a proportion of patients experience prolonged ill-health commonly known as "long-COVID", the nature of which remains to be fully characterised [10]. Larger studies are now needed to determine if persistently elevated plasma ACE2 activity may identify people at risk of prolonged illness or cardiac events following COVID-19 infection, as well as studies that address whether therapeutic strategies directed at a dysregulated RAS can reduce COVID-19 complications.

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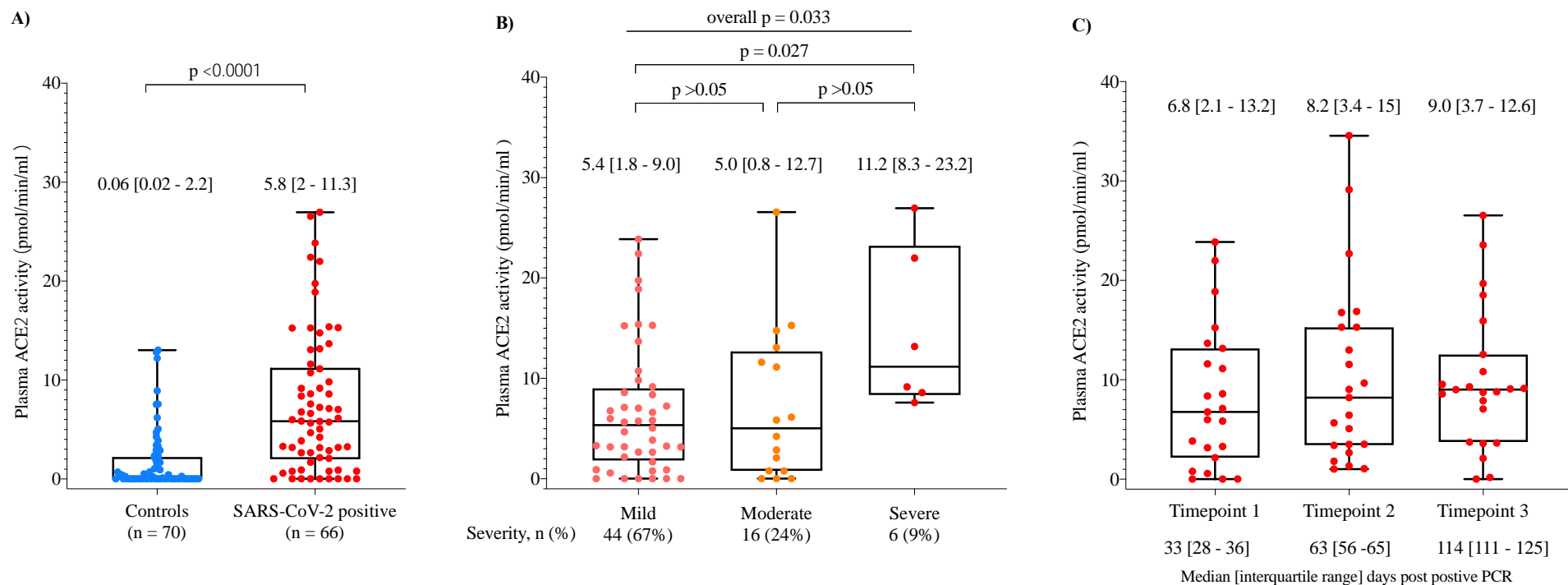
Figure 1: Plasma ACE2 activity is elevated in patients who recovered from SARS-CoV-2 infection (A), is increased with disease severity (B), and remains elevated 2-3 months after SARS-CoV-2 infection.

Data shown as scatter points and box plot with whiskers representing min and max values of median plasma ACE2 activity between controls, and patients who recovered from SARS-CoV-2 infection (A), and median plasma ACE2 activity in patients recovered from SARS-CoV-2 infection according to disease severity (B). The median [interquartile range] plasma ACE2 activity levels are shown above each bar. The median plasma ACE2 activity in patients recovered from SARS-CoV-2 infection with serial samples (n=23) are shown in (C). The median [interquartile range] plasma ACE2 activity levels are shown above the bar for each timepoint. Two-tailed P-values <0.05 were considered significant.