Reply to Sun *et al.*

From the Authors:

We thank Sun and colleagues for their interest in our recent work published in the *Journal* (1). In short, we found lower circGSAP (circular RNA–γ-secretase–activating protein) levels were associated with increased occurrence and poorer outcomes in patients with idiopathic pulmonary arterial hypertension (IPAH), which means that circGSAP may be an emerging biomarker for the diagnosis and prognosis of IPAH. As commended by Sun and colleagues, this is an important field, and the impact of circular RNAs (circRNAs) on the diagnosis and treatment of IPAH has not yet been fully studied. Here, we answer comments raised by Sun and colleagues.

We agree with Sun and colleagues on their suggestion to list *P* values for comparisons of baseline information between healthy control subjects and patients with IPAH. Indeed, there was no significant difference in age, body mass index (BMI), and hazard ratio (HR) between the healthy control subjects and the patients with IPAH in the discovery group (age, $P = 0.790$; BMI, $P = 0.891$; HR, $P = 0.431$) or in the validation group (age, $P = 0.166$; BMI, $P = 0.112$; HR, $P = 0.183$). Although there was significant difference in sex between the healthy control subjects and the patients with IPAH in the validation group ($P = 0.023$), our results were accurate after adjusting for age, sex, and BMI in the multivariate regression analysis.

Sun and colleagues suggested building a model to predict IPAH through taking circGSAP and other renowned indicators into account. We acknowledged in our study that 6-minute-walk distance and NT-proBNP are reliable indicators for the prognosis of IPAH (1). It is believed that an optimal model can be built to predict the diagnosis and prognosis of patients with IPAH by including circGSAP, 6-minute-walk distance, NT-proBNP, or even other hemodynamic parameters. To highlight the impact of circGSAP on IPAH, only the receiver operating curve area of circGSAP was shown in our manuscript.

We cannot agree more with Sun and colleagues on their suggestion to expand the sample size by recruiting patients from multicenters. We have collected the plasma and peripheral blood mononuclear cells of patients with pulmonary hypertension (PH) due to other etiologies in our center to validate our findings from patients with IPAH. Furthermore, we have drafted a plan to run a multicentered prospective study to test the downregulation of circGSAP in PH.

Finally, Sun and colleagues suggested to summarize signaling pathways related to circGSAP for facilitating future research on potential mechanisms and therapeutic targets. It is known that circRNAs are covalently closed, abundant, and evolutionarily conserved. Many of them are involved in important biological functions by acting as microRNA sponges, binding to RNA-binding proteins, regulating mRNA transcription, expression, or translating into proteins (2–6). In our study, we found that circGSAP was an exonic circRNA and could serve as microRNA sponges to regulate mRNA in the development of IPAH (1). We are studying its downstream signaling pathways regarding the proliferation or apoptosis of endothelial cells of the pulmonary arteries both *in vivo* and *in vitro*. This work is near completion and our results will be presented in the next publication. It is also our hope to find novel therapeutic targets through elaborating the functional characteristics of circGSAP in the pathogenesis of PH. ■

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High Circulating Plasma Soluble Receptor for Advanced Glycation End-Products in Early COVID-19–associated Acute Respiratory Distress Syndrome: Pathophysiological Significance?

To the Editor:

I read with interest the article by Kapandji and colleagues (1) arguing in favor of the early intense lung alveolar epithelial injury as the

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predominant pathophysiology of coronavirus disease (COVID-19)–associated acute respiratory distress syndrome (CARDS) based on marked elevation in the s-RAGE (soluble form of the receptor for advanced glycation end-products) levels among patients with CARDS. I concur with the authors' conclusion that plasma levels of s-RAGE correlate with CARDS severity and that the higher average plasma s-RAGE level in patients with CARDS in comparison with non-CARDS (1) strongly implies key differences in the early pathophysiological process in these subsets of ARDS. However, despite significantly greater average baseline s-RAGE levels observed in CARDS in comparison with non-CARDS, a closer look into the data produced by Kapandji and colleagues revealed that a higher proportion of patients in the non-CARDS group had severe ARDS (45% vs. 26%) and a higher ventilatory ratio. Furthermore, the average Pa_{O_2}/FI_{O_2} ratio was significantly lower in the non-CARDS group than in the CARDS group. Thus, the higher baseline s-RAGE levels may not simply imply more severe lung epithelial injury in the CARDS group versus the non-CARDS group. Based on this argument, I believe, an alternate source for higher plasma s-RAGE levels early in the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection could be a possibility. Considering the fact that a higher proportion of patients in the CARDS group had systemic disorders such as hypertension, diabetes, and at least one cardiovascular risk factor, the greater Day 90 mortality observed in the CARDS group (1) may be secondary to the nonpulmonary causes. Importantly, COVID-19 is a multisystem disorder with histopathological evidence in favor of early endothelial injury even before any significant epithelial injury (2, 3). So, an important question that arises is whether an early endothelial injury is responsible for higher baseline s-RAGE levels observed in the CARDS group and thus describes a pathophysiological phenomenon unique to the atypical CARDS.

It has been postulated that RAGE plays a role in the SARS-CoV-2–mediated inflammatory response in the lungs (2, 4, 5). When healthy, the lung epithelial cells, apart from skin, are the only cell types known to express RAGE (5). However, in inflammatory disorders, increased expression of RAGE may occur on the immune cells such as monocytes and dendritic cells as well as on the endothelial cells (4, 5). Upregulation of RAGE on the endothelial cells is known to play an important role in the pathophysiology of vascular dementia, uncontrolled diabetes, coronary artery disease, and obesity, the known risk factors for COVID-19 (5). Ang II (angiotensin II), tumor necrosis factor-α, and IL-1β levels can induce expression of RAGE on the endothelial cells (5), and an increase in these inflammatory mediators was hypothesized as the cause of the complex clinical picture of COVID-19 (2, 4, 5). Thus, activation of the ACE (angiotensin-converting enzyme)/Ang II/AT1R (Ang II type 1 receptor) pathway after the binding of SARS-CoV-2 to ACE2 via its S-protein (spike protein) to invade host cells can result in RAGE activation (3) on the pulmonary epithelial as well as endothelial cells. Furthermore, activation of ADAM 17 (a disintegrin and metalloprotease 17), directly after SARS-CoV-2 S-protein binding to ACE2 (6), and indirectly by Ang II–p38-MAPK (p38 mitogen-activated protein kinase) axis (7) and RAGE–p38-MAPK pathway (8), can result in excessive ectodomain shedding of RAGE, resulting in high plasma s-RAGE levels in early COVID-19. Interestingly, in a study by Kehribar and colleagues, the serum s-RAGE level was significantly higher in the asymptomatic COVID-19 group than in the control group (9). In addition, after adjusting for age, serum s-RAGE level was higher in the patients with lung involvement than in the control group and the asymptomatic COVID-19 group. Based on the above arguments and from Kapandji and colleagues' observation of differences in baseline s-RAGE levels between COVID-19 and non–COVID-19 pneumonia, I postulate that early dysregulation of the renin–angiotensin system and ADAM-17 overactivation are the processes distinct to the early phase of SARS-CoV-2 infection that can result in transactivation of RAGE axis and early increase in s-RAGE. This phenomenon may not simply mirror the severity of lung epithelial injury; instead, it may suggest indirect endothelial dysfunction. I further propose Kapandji and colleagues perform subgroup analysis of their data to compare the plasma s-RAGE levels in patients with mild COVID-19 disease, patients with mild non-CARDS, and healthy control subjects as this could further help in understanding the phenomenon of epithelial–endothelial cross-talk in COVID-19 (2).

Much evidence suggests that endothelial injury augments RAGE expression and the amount of circulating s-RAGE reflects RAGE expressed on injured endothelial cells (10). In severe sepsis, the serum level of s-RAGE was strongly associated with that of vascular cell adhesion molecule-1, an early marker of endothelial activation related to systemic inflammation (10) and COVID-19 (11). Importantly, the histopathological findings of distinctly higher incidence of pulmonary microthrombi, as high as 73% in COVID-19 as compared with H1N1 influenza (24%), emphasizes the importance of extensive endothelial dysfunction in COVID-19 (12).

In conclusion, early ADAM-17–mediated ectodomain shedding of RAGE, endothelial activation, and inflammation may be contributory to the high baseline s-RAGE levels in patients with CARDS in the study of Kapandji and colleagues. Finally, measurements of specific markers for alveolar epithelial cell injury (human-type I cell 56-kD protein and cytokeratin 18), marker of alveolar capillary barrier disruption (surfactant protein D), and endothelial injury such as angiopoietin 2 may provide distinct temporal characteristics of circulating alveolar epithelial and endothelial injury markers in COVID-19. ■

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Reply to Jain

From the Authors:

We thank Dr. Jain for his interest in our study (1). In his comment, he raises the issue of an alternate source of s-RAGE (soluble form of the receptor for advanced glycation end-products) in patients with coronavirus disease (COVID-19). More specifically, he suggests a key role of endothelial injury and ACE2 (angiotensin-converting enzyme 2)/ADAM17/TMPRSS2 pathway to explain baseline differences in levels of plasma s-RAGE between COVID-19–associated acute respiratory distress syndrome (CARDS) and non-CARDS. This could be of importance as this would suggest the role of systemic aggression in the morbidity and mortality associated with COVID-19 infection, may support a different pathophysiology of CARDS, and would limit the interpretation of s-RAGE as a marker of alveolar aggression in this subgroup.

As our study was not designed for that purpose, it is difficult to answer precisely to this assertion. However, Jain correctly points out some imbalance between CARDS and non-CARDS groups, which could explain observed baseline levels differences, particularly through an endothelial production of s-RAGE.

First, we observed a higher prevalence of cardiovascular comorbidities in CARDS. We strongly agree that the level of soluble S-RAGE increases in inflammation, vascular dementia, diabetes, cardiovascular disease, and obesity. Nevertheless, in published data, s-RAGE remains below 1,000 pg/ml in these pathological conditions. In ARDS, plasma s-RAGE levels are between 3,000 and 4,000 pg/ml and bronchoalveolar samples show higher levels (up to 400,000 pg/ml) related to S-RAGE production by lung type 1 cells (2). It is therefore unlikely that the amount of s-RAGE related to comorbidities may have influenced our findings.

We agree that the AGE–RAGE axis is dysregulated in patients with diabetes or obesity, predisposing them to severe COVID-19 forms (3). If the cross-talk between Ang II/AT1R and RAGE after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection could explain the pulmonary lesions observed in CARDS (increased lung capillary permeability and epithelial and endothelial damages), the predominance of endothelial lesions over pulmonary epithelial lesions in this context is not well established (4). From a clinical perspective, despite a high reported rate of thrombotic events (5), early mortality is mainly explained by refractory hypoxemia (78% in our cohort).

Second, Jain points out the lower proportion of severe ARDS in the CARDS group than the non-CARDS group, despite higher baseline s-RAGE levels suggesting that these results do not reflect increased lung epithelial injury. Our data did not support this assertion: when comparing s-RAGE levels in patients with mild ARDS, we did not observe any significant difference between patients with COVID-19 and patients without COVID-19 (2,217 pg/ml [1,802–3,545] vs. 1,594.5 pg/ml [1,113.7–2,658.4]; $P = 0.277$). In contrast, s-RAGE levels were significantly higher in patients with COVID-19 with moderate or severe ARDS (data not shown). s-RAGE levels in both patients with COVID-19 and without COVID-19 significantly differed from control subjects (525.0 pg/ml [411.0–638.5]; $P < 0.001$) regardless of ARDS severity.

We believe that other factors could explain these differences. Several studies have reported that lung imaging patterns are associated with distinct profiles of lung injury biomarkers (including s-RAGE) during ARDS (6). We therefore compared s-RAGE levels according to lung morphology on imaging. Patients were classified as presenting focal pattern if areas of lung attenuation had lobar or segmental distribution, and nonfocal pattern if lung attenuations were diffusely distributed throughout the lung (7).

According to this definition, all patients with CARDS had a nonfocal radiological pattern. In patients with non-CARDS, 31 had focal and 86 nonfocal patterns. s-RAGE was significantly higher in patients with CARDS than those with non-CARDS with focal pattern (4,044.0 pg/ml [1,763.0–4,768.0] vs. 876.9 pg/ml [516.8–1,009]; $P < 0.001$) but did not differ from those with nonfocal pattern (4,044.0 pg/ml [1,763.0–4,768.0] vs. 3,074 pg/ml [1,933–4,375]; $P = 0.29$). Interestingly, taking into account radiological pattern, mortality was higher in patients with CARDS than in focal ARDS (adjusted hazard ratio, 2.58 [1.01–6.63]). This difference was not significant when compared with nonfocal ARDS (1.35 [0.77–2.35]).

In conclusion, we cannot rule out the possibility that s-RAGE levels in CARDS and non-CARDS may have been influenced by extrapulmonary epithelial factors, such as endothelium damages. If

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