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CORRESPONDENCE

Increase in serum levels of phosphatidylserine-specific phospholipase A_1 in COVID-19 patients

Takuya Shimura[1,6], Makoto Kurano[1,2,6]✉, Koh Okamoto[3], Daisuke Jubishi[3], Kuniyuki Kano[4], Koji Igarashi[5], Satoshi Shimamoto[5], Junken Aoki[4], Kyoji Moriya[3] and Yutaka Yatomi[1,2]✉

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The development of novel drugs to overcome the current global coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an important task. Although antiviral agents have been investigated, one of the important goals of treatment is also to control the biological response to the infection. A series of elegant basic studies have revealed that lysophosphatidylserine (LysoPS) might play important roles in inflammation through three kinds of G protein-coupled receptors [1]; LysoPS reportedly suppresses activation of T cells [2] and secretion of inflammatory cytokines from macrophages [3] and promotes phagocytosis of apoptotic cells, including apoptotic neutrophils, by macrophages [4]. Considering these proposed physiological effects, it appears that LysoPS might have important roles in the resolution of inflammation. Phosphatidylserine-specific phospholipase A_1 (PS-PLA_1) has been proposed to be involved in the production of LysoPS [5].

In the present study, we investigated the serum levels of PS-PLA_1 in 58 healthy adult volunteers and 133 COVID-19 patients, consisting of 127 symptomatic patients and 6 asymptomatic patients. The 127 symptomatic COVID-19 patients were classified into three groups according to disease severity: severity level 1 (mild disease, did not require oxygen therapy), severity level 2 (moderate disease, required oxygen therapy but not mechanical ventilatory support), and severity level 3 (severe disease, required mechanical ventilatory support). The PS-PLA_1 levels were determined by a two-site immunoenzymometric assay with the TOSOH AIA system (TOSOH, Tokyo, Japan) [6]. The method is described in detail in the Supplementary Materials and Methods section.

Compared to those in the healthy group, as shown in Fig. 1A, the serum PS-PLA_1 levels were consistently and significantly higher during the clinical course, each day after the onset of symptoms, in the COVID-19 patients. From 7 COVID-19 patients, we collected serum samples 2–11 days prior to the onset of symptoms. The time course, shown in Fig. 1B, of the serum PS-PLA_1 levels showed that the levels tended to increase on days 6–7 after the onset of symptoms ($P = 0.07$). The serum PS-PLA_1 levels were also significantly higher from days 3–4 to days 11–12 than the levels measured after day 21 from symptom onset, suggesting that the serum PS-PLA_1 levels did increase specifically in response to the infection in patients with symptomatic COVID-19. The time course of the serum PS-PLA_1 levels in the asymptomatic subjects is shown in Supplementary Fig. 1. Comparison of the PS-PLA_1 levels in the serum samples collected on days 1–2 to days 9–10 ($n = 16$) from the six asymptomatic patients with those in the serum samples from healthy subjects ($n = 58$) revealed that the serum PS-PLA_1 levels were significantly higher in the asymptomatic COVID-19 patients than in the healthy control subjects ($P < 0.01$).

To date, elevated serum PS-PLA_1 levels have been noted only in a limited number of pathological states, including cancers, SLE, and hyperthyroidism [7]. Among these, inflammation akin to that seen in SLE might possibly explain the increase in the serum PS-PLA_1 levels in COVID-19 patients since several phenotypes of COVID-19 are characterized by the presence of anti-phospholipid antibody, antinuclear antibody, and systemic endotheliitis at rather high frequencies, with the possible involvement of NETosis [8].

In regard to the association of elevated PS-PLA_1 levels with the severity and clinical parameters of COVID-19, in the subjects with mild COVID-19 (severity level 1), the serum PS-PLA_1 levels were significantly higher than the levels measured after day 21 from symptom onset (Fig. 1C). In the patients with moderate COVID-19 (severity level 2), the serum PS-PLA_1 levels were also higher than those measured after day 21 from symptom onset (Fig. 1D). In contrast, in the patients with severe COVID-19 (severity level 3), no significant elevation of the serum PS-PLA_1 levels was observed (Fig. 1E). The PS-PLA_1 levels were found to be higher in the patient group with severity level 2 than in the patient group with severity level 1 on days 7–8 and higher than those in the patient group with severity level 3 on days 7–8 and days 11–12 (Fig. 1F). As shown in Supplementary Fig. 2 and Supplementary Table 1, the serum PS-PLA_1 levels showed significantly positive correlations with the serum CRP levels but a significantly negative correlation with the serum D-dimer levels on days 13–14. The serum PS-PLA_1 levels showed significant negative correlations with the anti-SARS-CoV-2 IgM titers measured on days 11–12 and anti-SARS-CoV-2 IgG levels measured from days 11–12 to days 15–16.

Considering that LysoPS might have important roles in the resolution of inflammation [2–4], together with the result that the serum PS-PLA_1 levels were lower in patients with severe COVID-19 than in those with moderate COVID-19 (Fig. 1C–F), we propose the hypothesis that failure of the serum PS-PLA_1 levels to increase adequately to suppress an overreactive immune system could

[1]Department of Clinical Laboratory, The University of Tokyo Hospital, Tokyo, Japan. [2]Department of Clinical Laboratory Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan. [3]Department of Infectious Diseases, The University of Tokyo Hospital, Tokyo, Japan. [4]Department of Health Chemistry, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan. [5]Bioscience Division, TOSOH Corporation, Kanagawa, Japan. [6]These authors contributed equally: Takuya Shimura, Makoto Kurano. ✉email: kurano-tky@umin.ac.jp; yatoyuta-tky@umin.ac.jp

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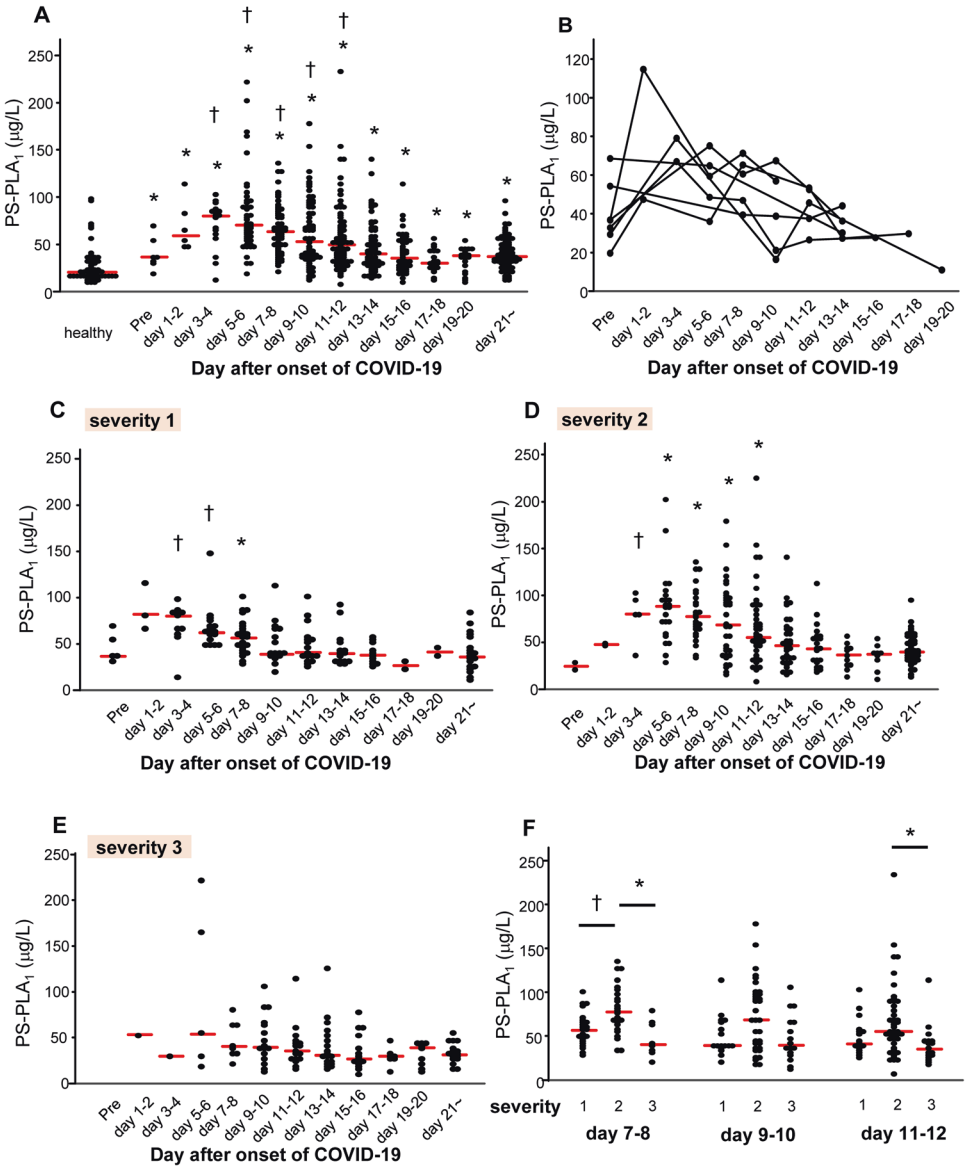

Fig. 1 Serum PS-PLA$_1$ levels in subjects with COVID-19. The serum PS-PLA$_1$ levels were measured in patients with COVID-19 (severity level 1, $n = 41$; severity level 2, $n = 62$; severity level 3, $n = 24$) and healthy subjects ($n = 58$). **A** Time course of serum PS-PLA$_1$ levels in symptomatic COVID-19 patients and distribution of serum PS-PLA$_1$ levels in healthy subjects. The differences in the levels between the healthy subjects and COVID-19 patients were assessed by the Mann–Whitney *U* test, $^*P < 0.01$ vs. healthy subjects. Differences between the serum PS-PLA$_1$ levels measured on specified days after the onset of COVID-19 symptoms and those measured after day 21 from symptom onset in individual subjects were assessed by the Wilcoxon signed-rank sum test, $^{\dagger}P$ 0.01 vs. level measured after day 21. **B** Time course of the serum PS-PLA$_1$ levels in the COVID-19 patients for whom samples collected before disease onset (Pre) were available ($n = 7$). **C**–**F** Time course of serum PS-PLA$_1$ levels in patients with mild (**C**), moderate (**D**), and severe COVID-19 (**E**). $^*P < 0.01$; $^{\dagger}P < 0.05$ vs. level measured after day 21 from symptom onset. **F** Differences in the serum PS-PLA$_1$ levels on days 7–8, days 9–10, and days 11–12. The differences were assessed using an independent Kruskal–Wallis test, followed by the Games Howell test for post hoc analysis. $^*P < 0.01$; $^{\dagger}P < 0.05$. The horizontal bars represent the means of independent samples

result in the development of severe COVID-19 as a result of a cytokine storm. The negative association with the serum D-dimer levels might be consistent with the serum PS-PLA$_1$ levels being lower in patients with severe COVID-19 than in those with moderately severe disease, while the positive correlation with the serum CRP levels might be consistent with the elevated serum PS-PLA$_1$ levels declining faster in patients with mild COVID-19 than in those with moderate COVID-19. PS-PLA$_1$ is expressed in immune cells, such as dendritic cells, T cells, and macrophages, and in various tissues, including the lung and liver [7]. Considering these origins, a possible mechanism of modulation of the serum PS-PLA$_1$ level in a bell-shaped manner depending on the severity of COVID-19 might be impaired upregulation of PS-PLA$_1$ expression in immune cells in severe COVID-19 patients, resulting in inappropriate immune responses, and/or the severely injured lungs and/or liver in severe COVID-19 failing to maintain adequate serum PS-PLA$_1$ levels. Interestingly, the serum PS-PLA$_1$ levels were negatively correlated with the serum anti-SARS-CoV-2 antibody levels. Considering that LysoPS plays important roles in the biology of lymphocytes [2], PS-PLA$_1$ might affect the generation of anti-SARS-CoV-2 antibodies through LysoPS.

In summary, COVID-19 patients showed elevated serum levels of PS-PLA$_1$, an enzyme involved in the synthesis of LysoPS, in a bell-shaped manner depending on the severity of COVID-19. The

alteration of the serum PS-PLA$_1$ levels might represent compensatory biological responses directed at suppressing immunological overreaction of the body in COVID-19, which is an important risk factor for mortality from the disease.

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AUTHOR CONTRIBUTIONS

Conceptualization: MK, JA, KM, and YY. Methodology: TS, SS, and KI. Investigation: TS, MK, KO, DJ, and KK. Visualization: TS, MK. Funding acquisition: MK, JA, and YY. Project administration: MK, KO, DJ, KM, and YY. Supervision: MK, YY. Writing—original draft: MK. Writing—review and editing: KO, DJ, KK, KI, JA, KM, and YY.

COMPETING INTERESTS

KI and RS are employees of TOSOH Corporation.

ADDITIONAL INFORMATION

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Correspondence and requests for materials should be addressed to M.K. or Y.Y.

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