Relationship between serum soluble endothelial protein C receptor level and COVID-19 findings

Nergiz Bayrakci[a], Gulsum Ozkan[a], Levent Cem Mutlu[b], Iknur Erdem[c], Ilker Yildirim[d], Dumrul Gulen[e] and Aliye Celikkol[f]

Coronavirus-related disease-2019 (COVID-19)-associated coagulopathy presents predominantly with thrombosis and leads to complications in close association with inflammatory process. Soluble endothelial protein C receptor (sEPCR), which is the soluble form of EPCR, reduces the anticoagulant and anti-inflammatory activity of activated protein C. The purpose of this study is to investigate the relationship between sEPCR and the laboratory parameters and thorax computed tomography (CT) findings in the course of COVID-19. Twenty-five laboratory-confirmed [reverse transcription-quantitative polimerase chain reaction (RT-qPCR) positive] and 24 clinically diagnosed (RT-qPCR negative) COVID-19 patients were enrolled in the study. Blood specimens were collected for sEPCR and haematological and biochemical parameter measurement. Thorax CT was performed to detect COVID-19 findings. These parameters from RT-qPCR positive and negative patients were then compared. Although there was no difference between the groups in terms of symptoms, the time between the onset of symptoms and the admission time was shorter in RT-qPCR positive group ($P = 0.000$). sEPCR levels were significantly higher in the RT-qPCR positive group ($P = 0.011$). Patients with ground-glass opacity and bilateral involvement on thorax CT have higher serum sEPCR levels ($P = 0.012$ and 0.043, respectively). This study has shown for the first time that serum sEPCR levels, which is a member of coagulation cascade and has also been reported to be associated with inflammation, is higher in patients with positive RT-qPCR test and patients with GGO or bilateral involvement on thorax CT regardless of the PCR result. *Blood Coagul Fibrinolysis* 32:550–555

Blood Coagulation and Fibrinolysis 2021, **32**:550–555

Keywords: coagulopathy, COVID-19, endothelial damage, pneumonia, soluble endothelial protein C receptor

[a]Department of Nephrology, [b]Department of Pulmonary Medicine, [c]Department of Infectious Diseases, [d]Department of Anesthesiology and Reanimation, [e]Department of Microbiology and [f]Department of Clinical Biochemistry, Tekirdag Namik Kemal University, School of Medicine, Tekirdag, Turkey

Correspondence to Nergiz Bayrakci, MD, Tekirdag Namik Kemal University, School of Medicine, Department of Nephrology, 59030 Tekirdag, Turkey
Tel: +905058879273; e-mail: nbayrakci@nku.edu.tr

Received 23 April 2021 **Revised** 1 July 2021
Accepted 9 July 2021

Introduction

Coronavirus-related disease-2019 (COVID-19), which caused by severe acute respiratory distress syndrome coronavirus 2 (SARS-CoV-2), is a systemic infectious disease with a wide spectrum of symptoms and signs. The first case was reported in December 2019 and has become a pandemic as of March 2020 [1]. The most widespread and only direct method for determining SARS-CoV-2 for diagnosis of COVID-19 is reverse transcription-quantitative polimerase chain reaction (RT-qPCR) performed mostly on nasopharyngeal swab, has a reported sensitivity of 63–78%. RT-qPCR test might give false-negative result because of testing too early (due to low viral load) or too late (due to viral load displacement), inappropriate or insufficient sample and improper transportation and storage of the sample [2–4]. Therefore, in symptomatic patients with negative RT-qPCR test, thorax computed tomography (CT) findings guide the diagnosis and may prevent delay in diagnosis and treatment of COVID-19 [5].

The host receptor that mediates the entry of SARS-CoV-2 into the cell is angiotensin-converting enzyme 2'dir (ACE2) as in SARS-CoV. SARS-CoV-2 binds to ACE2 through the receptor-binding gene region of its spike protein [6,7]. A rapid cytokine response occurs following the entry of the virus into the cell followed by e series of complications such as cytokine release syndrome, thromboembolism and acute respiratory distress syndrome (ARDS). It is known that thromboembolic complications can occur at any age and without any risk factor [8–12]. This process, also called 'COVID-19 associated coagulopathy', has not been clearly identified. The main laboratory findings are, normal or slightly prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT); high d-Dimer and fibrinogen levels and variable platelet level, which are consistent with infection-induced inflammatory changes [13–15].

Protein C system is an important member of primary anticoagulant system, wherein FVIIa and FVa activity regulated thorough activated protein C (APC). Thrombin-thrombomodulin complex activates protein C to produce APC. Subsequently, endothelial protein C receptor (EPCR), a transmembrane protein localized endothelial

0957-5235 Copyright © 2021 Wolters Kluwer Health, Inc. All rights reserved. DOI:10.1097/MBC.0000000000001070

cell, accelerates this activation. In addition to its anticoagulant effects, APC has anti-inflammatory and cytoprotective effects. The effects of APC on leukocyte and the other cell functions involved in inflammatory response take place via EPCR [16–18]. Soluble EPCR (sEPCR), resulting from proteolysis of ERCP, inhibits APC-phospholipid bindings and causes APC dysfunction. This process is strictly regulated by inflammatory and coagulant factors [19]. There are several studies reporting high sEPCR levels in some disease accompanied by coagulopathies such as systemic lupus erythematosus (SLE), preeclampsia, malaria and that this is associated with poor prognosis, severe pneumonia and ARDS [20–24].

The purpose of this study is to investigate the relationship between sEPCR level and COVID-19 findings, which is frequently accompanied thromboinflammatory complications.

Materials and methods

Twenty-five laboratory-confirmed (RT-qPCR positive) and 24 clinically diagnosed [RT-qPCR negative) based on chest CT and clinical findings] COVID-19 patients over the age of 18 years admitted to Tekirdag Namik Kemal University Hospital, were included. Exclusion criteria were pregnancy, using anticoagulant or antiaggregant, hepatic disease and systemic infection. Patients were enrolled once the local ethical committee had confirmed that the study protocol was compatible with the second Declaration of Helsinki. Patients underwent detailed physical examinations, and their demographic data and vital findings were recorded. Nasopharyngeal swab specimens were collected for the COVID-19 RT-qPCR test. Repeat RT-qPCR specimens were collected after 48 h from patients with negative swab results. Chest CT was performed for the diagnosis of COVID-19 pneumonia. Patients were subsequently divided into laboratory-confirmed (RT-qPCR positive) and clinically diagnosed (RT-qPCR negative, thoracic CT and clinical findings positive) depending on their nasal swab specimen results. Blood specimens (10 ml) were collected from all patients immediately on hospitalization from a large vein in the antecubital region between 08.00 and 10.00 a.m. after 12-h fasting. Serum biochemistry, complete blood count and coagulation parameters measurements were performed on the same day. Blood specimens obtained for sEPCR measurement were immediately centrifuged for 10 min at 2500 x *g* for serum collection. Serum specimens were then stored at -80°C until the day of study.

Measurement of soluble endothelial protein C receptor levels

Human sEPCR levels were measured using a Bioassay Technology Laboratory (Shanghai Korain Biotech Co. Ltd. Shanghai, China) commercial ELISA kit (catalogue no. E1245Hu, sensitivity 0.25 ng/ml, intra-assay variation coefficient $< 8\%$, inter-assay CV $< 10\%$).

SARS-CoV-2 RT-qPCR test

RT-qPCR tests involving oropharyngeal and nasopharyngeal swab specimens from patients presenting to the hospital on suspicion of COVID-19 infection were performed at the Medical Microbiology Laboratory. The swab specimens were placed into tubes containing 2–3 ml vNAT buffer. Viral RNAs were extracted in vNAT buffer with no additional extraction procedure. Amplification of open reading frame 1ab (ORF1ab) and nucleocapsid protein (N) target genes was performed using appropriate SARS-CoV-2 Double Gene RT-qPCR kits (Bioeksen R&D Technologies Ltd., Istanbul, Turkey) in line with the manufacturer's instructions. Reaction mixtures were prepared to a total volume of 20 μl with the addition of 10 μl 2X Prime Script Mix, 5 μl CVD Di Oligo Mix and 5 μl template nucleic acid. RT-qPCR tests were performed with reverse transcriptional reaction for 5 min at 52°C, predenaturation at 95°C for 10 s, 40-cycle denaturation at 95°C for 1 s and 40 cycle extension at 55°C for 30s, and fluorescence signal collection. Nonsigmoidal curves were defined as negative, while cycle threshold values (Cq) less than 38 were regarded as positive test results. Repeat specimens were requested for samples with Cq at least 38, and the RT-qPCR tests were repeated.

Thorax computed tomography findings

Thorax CT findings classified according to lesion type [ground glass opacity (GGO), consolidation, tree-in-bud, nodule, reticular density, lymphadenopathy, pleural effusion] and lesion localization (bilateral, unilateral).

Statistical analysis

Compatibility with normal distribution was evaluated using the Kolmogorov–Smirnov test. The *t*-test was applied in the comparison of normally distributed data, and the Mann–Whitney *U* test for nonnormally distributed data. The Chi-square test was employed in the evaluation of demographic variables, and Pearson correlation analysis for determining correlations. *P* values less than 0.05 were regarded as statistically significant.

Results

Demographic features, laboratory and computed tomography findings

Patients diagnosed with COVID-19 were compared in two groups: 24 patients in RT-qPCR negative group and 25 patients in RT-qPCR positive group. There was no difference in terms of age and sex between the groups. History of contact with an infected person was more frequent in RT-qPCR positive group ($P = 0.002$). Although there was no difference between the groups in terms of symptoms, the time between the onset of symptoms and the admission time was shorter in

Table 1 Baseline characteristics and laboratory findings of the study population

Characteristics	RT-qPCR negative ($n=24$) Mean (SD) or median (IQR) or *n* (%)	RT-qPCR positive ($n=25$) Mean (SD) or median (IQR) or *n* (%)	*P*
Age (years)	52 ± 18.	49 ± 15	0.501
Sex (female/male)	11/ 13	14 / 11	0.477
Symptom duration (day)	4 (1–20)	2 (0–12)	0.000
Contact (+/–)	2/22	12/13	0.002
Glucose (mg/dl)	128.79 ± 39.42	133.88 ± 61.96	0.405
Creatinine (mg/dl)	0.88 (0.48–8.94)	0.86 (0.61–1.64)	0.509
AST (IU/l)	25.90 (10–193)	27.90 (11–66)	0.711
ALT (IU/l)	19.55 (3–83)	21 (9–55)	0.617
LDH (IU/l)	256.61 ± 74.62	244.91 ± 67.62	0.870
Albumin (g/dl)	4.36 (2.3–5)	4.27 (1.2–4.9)	0.59
CRP (mg/l)	40.4 (7.54–223.7)	11.9 (0.78–160.34)	0.03
Procalcitonin (ng/ml)	0.157 (0.6–154)	0.060 (0.02–0.310)	0.001
Ferritin (ng/ml)	139.55 (14–1202)	147.8 (14–826)	0.762
Sedimentation (mm/h)	44.58 ± 28.87	33.40 ± 24.54	0.340
Haemoglobin (g/dl)	13.1 ± 2.5	13.4 ± 2.02	0.348
WBC ($10^3/\mu l$)	8.94 (3.69–22.36)	4.97 (3.35–9.03)	0.001
Neutrophile ($10^3/\mu l$)	5.55 (2.51–14.9)	3.22 (1.6–6.87)	0.002
Lymphocyte ($10^3/\mu l$)	1.71 (0.4–5.08)	1.2100 (0.19–2.24)	0.18
Monocyte ($10^3/\mu l$)	0.65 (0.28–2.06)	0.43 (0–0.98)	0.016
Platelet ($10^3/\mu l$)	212 (73–466)	196 (116–344)	0.322
Fibrinogen (mg/dl)	436.59 ± 142.91	340.80 ± 131.03	0.502
D-dimer (mg/l)	0.80 (0.19–35.2)	0.53 (0–1.88)	0.051
INR	1.10 ± .053	1.05 ± 0.09	0.022
aPTT (s)	24.61 ± 2.91	24.71 ± 2.79	0.799
sEPCR (ng/ml)	9.35 (5.69–14.12)	10.35 (7.03–68)	0.011

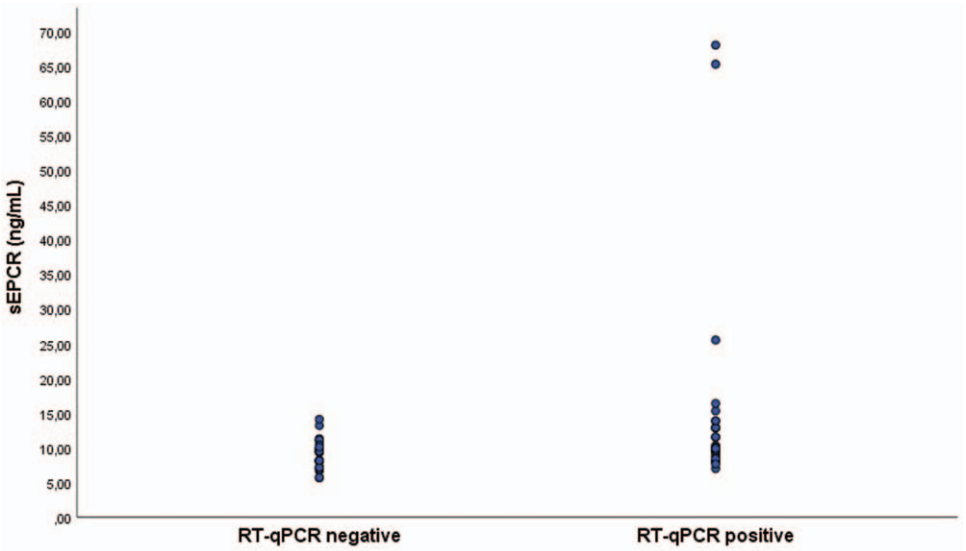
ALT, alanine aminotransferase; aPTT, activated partial thromboplastin time; AST, aspartate aminotransaminase; CRP, C-reactive protein; INR, International normalization ratio; LDH, lactate dehydrogenase; RT-qPCR, real-time reverse transcription–PCR; sEPCR, soluble endothelial protein C receptor; WBC, white blood cell count. *P* value < 0.05 is considered to be significant.

RT-qPCR positive group ($P=0.000$). Procalcitonin, C-reactive protein (CRP), leukocyte and international normalization ratio (INR) levels were higher in RT-qPCR negative group ($P=0.001$; 0.003; 0.001; 0.022, respectively) (Table 1). All patients had CT findings in favour of COVID-19. The two most common signs were GGO and consolidation (77.6 and 40.8%, respectively). Bilateral involvement was detected in 66.7% of the patients.

Soluble endothelial protein C receptor levels

sEPCR levels were 9.35 (5.68–14.11) ng/ml in the RT-qPCR negative group and 10.35 (7.0368) ng/ml,

Fig. 1



Serum soluble endothelial protein C receptor levels in RT-qPCR positive and negative patients. sEPCR, soluble endothelial protein C receptor; RT-qPCR, reverse transcription-quantitative polimerase chain reaction. P value = 0.011.

Table 2 The relationship between thorax computed tomography findings and soluble endothelial protein C receptor levels of the study population

CT finding	sEPCR (ng/ml) [median (IQR)]	*P*
GGO (+) ($n=38$)	10.96 (7.68–68)	
GGO (−) ($n=11$)	8,02 (6.69–25.52)	0.012
Bilateral ($n=33$)	11.33 (7.21–68)	
Unilateral ($n=16$)	8.3 (6.69–12.92)	0.043

CT, computed tomography; GGO, ground glass opacity; sEPCR, soluble endothelial protein C receptor. *P* value <0.05 is considered to be significant.

significantly higher in the RT-qPCR positive group ($P=0.011$) (Table 1) (Fig. 1).

There was no correlation between sEPCR and other laboratory parameters.

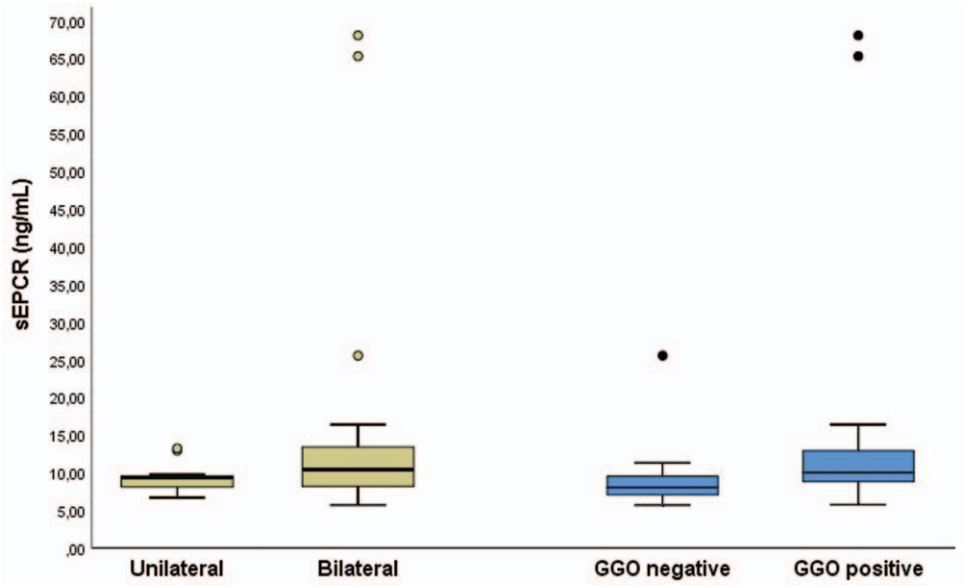
When the study group was evaluated in terms of CT findings, serum sEPCR levels were higher in patients with GGO and bilateral involvement ($P=0.012$; 0.043, respectively) (Table 2) (Fig. 2).

Discussion

EPCR plays a key role in initiation and maintenance of the anticoagulant and anti-inflammatory effects of APC, which produced from protein C through thrombin- thrombomodulin complex. sEPCR, which is the soluble form of EPCR and also found in healthy population, inhibits the activation of APC. High serum sEPCR levels have been reported in some disease such as SLE, cancer, stroke where inflammation and thrombosis are common [20–24].

The pathogenesis of SARS-Cov-2-associated hypercoagulability is not clearly understood. Major laboratory findings in this process, called thromboinflammation or COVID-19-associated coagulopathy, are normal or slightly prolonged PT and aPTT, variable levels of platelets, high levels of fibrinogen and d-Dimer. In addition, there are some studies reporting increased factor VIII activity, significantly elevated von-Willebrand factor level, slightly decreased antithrombin and protein S levels, slightly elevated protein C level [25]. It has been suggested that the direct effect of the virus disrupting endothelial integrity plays a key role in COVID-19 associated coagulopathy [15]. SARS-CoV-2 enters into the cell ACE2-mediated endocytosis. Intracellular changes following the endocytosis of the virus result in decreased nitric oxide production, aggravated vasoconstriction, increased leukocyte and platelet adhesion and finally increased thrombogenicity [26]. In this process, transmembrane protease serine 2 (TMPRSS-2) and tumour necrosis factor alpha converting enzyme (TACE), also called as A disintegrin and metalloprotease 17 (ADAM17) interact with ACE2 [7,27,28]. ADAM17 is a sheddase and is responsible for the extracellular breakdown of many transmembrane proteins and the release of their soluble forms into the circulation. ACE2, EPCR and tumour necrosis factor alpha (TNF alpha) are among the target proteins of ADAM17, which also takes place in sEPCR production. [29]. It has been reported that TNF alpha and interleukin 1-beta (IL-1beta) suppress serum EPCR level and simultaneously high sEPCR level was detected [30,31]. TNF alpha and IL-1beta are known to

Fig. 2



Serum soluble endothelial protein C receptor levels based on computed tomography findings. GGO, ground glass opacity; sEPCR, soluble endothelial protein C receptor.

be among the major cytokines in the cytokine storm seen in COVID-19 course [32]. Steven *et al.* [33] have suggested that considering the role of protein C system in inflammation and coagulation, APC may be involved in the treatment of disease based on the close relationship between inflammation and coagulation cascade in the course of COVID-19.

In our study, sEPCR levels were higher in RT-qPCR positive group. These patients were diagnosed earlier. The time between the onset of symptoms and admission time was shorter in this group. It is possible that sEPCR may increase suddenly with the onset of endothelial damage following virus entry in early stages of the disease. On the other side, major laboratory findings such as INR, leucocyte count, procalcitonin, CRP known to be consistent with disease progression and severity were higher and sEPCR levels were lower in RT-qPCR negative group. These patients were diagnosed later and they are more likely to have been detected at a later stage of the disease. Therefore, prominent findings may have varied. Considering all these issues it can be argued that sEPCR level increases in the early phase of the disease and then gradually decreases as some other parameters increase with the dynamic inflammatory response in the later stages of the disease.

Reported sensitivity and specificity for thorax CT findings in the diagnosis of COVID-19 are quite variable (60–98% and 25–53%, respectively). It is recommended in clinically suspicious but RT-qPCR negative patients [34]. In a study including 1014 patients, 308 of 403 RT-qPCR negative and clinically suspicious patients had CT findings in favour of COVID-19 (%75) [35]. Earliest and most common reported CT finding associated with COVID-19 is GGO and is usually bilaterally and peripherally located in lower zone [36]. Thorax CT was performed in all patients included in our study and COVID-19 associated findings were detected in all of them. Similar to the literature, the most common finding was GGO and bilateral involvement was found in most of the patients (77.6 and 66.7%, respectively). Regardless of the PCR results, patients with GGO and bilateral involvement had higher sEPCR levels than those without GGO and unilateral involvement. In a study including patients diagnosed with melioidosis-associated pneumonia, plasma sEPCR levels were found to be significantly higher and associated with high mortality. Authors suggested that high levels of sEPCR in these group could be secondary to TNF-alpha associated EPCR dysfunction [24]. Similar findings were reported in two different studies evaluating septic shock patients secondary to pneumococcal pneumonia and experimental malaria-associated ARDS model [21,22]. These findings support the view that there is an intersection between coagulation and inflammation cascade in systemic infectious diseases with severe lung involvement and that protein C system located at this junction. Therefore, our findings can be interpreted according to this implication.

In conclusion, COVID-19-associated coagulopathy is a complex process whose pathophysiology is not yet understood. Different mechanisms and mediators are involved in different stages of the disease. Close relationship between endothelial damage, coagulopathy and inflammation has been reported in many studies. This study is the first study investigating the relationship of sEPCR with thromboinflammatory process and pulmonary findings in the course of COVID-19. We believe that more detailed investigations of protein C and EPCR-related pathways may provide important information for the prevention and treatment of COVID-19-associated thromboembolic complications.

Acknowledgements

This study has not been presented or published previously.

The authors have no funding of report.

Conflicts of interest

There are no conflicts of interest.

References

1 Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, *et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**:497–506.
2 Zitek T. The appropriate use of testing for COVID-19. *West J Emerg Med* 2020; **21**:470–472.
3 Hong KH, Lee SW, Kim TS, Huh HJ, Lee J, Kim SY, *et al.* Guidelines for laboratory diagnosis of coronavirus disease 2019 (COVID-19) in Korea. *Ann Lab Med* 2020; **40**:351–360.
4 Loeffelholz MJ, Tang YW. Laboratory diagnosis of emerging human coronavirus infections: the state of the art. *Emerg Microbes Infect* 2020; **9**:747–756.
5 Fang Y, Zhang H, Xie J, Lin M, Ying L, Pang P, *et al.* Sensitivity of chest CT for COVID-19: comparison to RT-PCR. *Radiology* 2020; **296**:E115–E117.
6 Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; **579**:270–273.
7 Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, *et al.* SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 2020; **181**:271–280; e278.
8 Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention. *JAMA* 2020; **323**:1239–1242.
9 Klok FA, Kruip M, van der Meer NJM, Arbous MS, Gommers D, Kant KM, *et al.* Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res* 2020; **191**:145–147.
10 Merkler AE, Parikh NS, Mir S, Gupta A, Kamel H, Lin E, *et al.* Risk of ischemic stroke in patients with coronavirus disease 2019 (COVID-19) vs patients with influenza. *JAMA Neurol* 2020; **77**:1–7.
11 Helms J, Tacquard C, Severac F, Leonard-Lorant I, Ohana M, Delabranche X, *et al.* High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. *Intensive Care Med* 2020; **46**:1089–1098.
12 Khandait H, Gandotra G, Sachdeva S, Kramer CA, Nye D, Golamari R, *et al.* COVID-19 and hematology: what do we know so far? *SN Compr Clin Med* 2020; **2**:2631–2636.
13 Leticia de Oliveira Toledo S, Sousa Nogueira L, das Gracas Carvalho M, Romana Alves Rios D, de Barros Pinheiro M. COVID-19: review and hematologic impact. *Clin Chim Acta* 2020; **510**:170–176.
14 Bikdeli B, Madhavan MV, Gupta A, Jimenez D, Burton JR, Der Nigoghossian C, *et al.* Pharmacological agents targeting thromboinflammation in COVID-19: review and implications for future research. *Thromb Haemost* 2020; **120**:1004–1024.

15 Iba T, Connors JM, Levy JH. The coagulopathy, endotheliopathy, and vasculitis of COVID-19. *Inflamm Res* 2020; **69**:1181–1189.
16 Neyrinck AP, Liu KD, Howard JP, Matthay MA. Protective mechanisms of activated protein C in severe inflammatory disorders. *Br J Pharmacol* 2009; **158**:1034–1047.
17 Esmon CT. Crosstalk between inflammation and thrombosis. *Maturitas* 2004; **47**:305–314.
18 Van de Wouwer M, Collen D, Conway EM. Thrombomodulin-protein C-EPCR system: integrated to regulate coagulation and inflammation. *Arterioscler Thromb Vasc Biol* 2004; **24**:1374–1383.
19 Liaw PC, Neuenschwander PF, Smirnov MD, Esmon CT. Mechanisms by which soluble endothelial cell protein C receptor modulates protein C and activated protein C function. *J Biol Chem* 2000; **275**:5447–5452.
20 Saposnik B, Peynaud-Debayle E, Stepanian A, Baron G, Simansour M, Mandelbrot L, *et al.* Elevated soluble endothelial cell protein C receptor (sEPCR) levels in women with preeclampsia: a marker of endothelial activation/damage? *Thromb Res* 2012; **129**:152–157.
21 Dos Santos Ortolan L, Sercundes MK, Moura GC, de Castro Quirino T, Debone D, de Sousa Costa D, *et al.* Endothelial protein C receptor could contribute to experimental malaria-associated acute respiratory distress syndrome. *J Immunol Res* 2019; **2019**:3105817.
22 Chapelet A, Foucher Y, Gerard N, Rousseau C, Zambon O, Bretonniere C, *et al.* An early increase in endothelial protein C receptor is associated with excess mortality in pneumococcal pneumonia with septic shock in the ICU. *Crit Care* 2018; **22**:251.
23 Kurosawa S, Stearns-Kurosawa DJ, Carson CW, D'Angelo A, Della Valle P, Esmon CT. Plasma levels of endothelial cell protein C receptor are elevated in patients with sepsis and systemic lupus erythematosus: lack of correlation with thrombomodulin suggests involvement of different pathological processes. *Blood* 1998; **91**:725–727.
24 Kager LM, Schouten M, Wiersinga WJ, de Boer JD, Lattenist LC, Roelofs JJ, *et al.* Overexpression of the endothelial protein C receptor is detrimental during pneumonia-derived gram-negative sepsis (Melioidosis). *PLoS Negl Trop Dis* 2013; **7**:e2306.
25 Panigada M, Bottino N, Tagliabue P, Grasselli G, Novembrino C, Chantarangkul V, *et al.* Hypercoagulability of COVID-19 patients in intensive care unit: a report of thromboelastography findings and other parameters of hemostasis. *J Thromb Haemost* 2020; **18**:1738–1742.
26 Verdecchia P, Cavallini C, Spanevello A, Angeli F. COVID-19: ACE2centric infective disease? *Hypertension* 2020; **76**:294–299.
27 Haga S, Yamamoto N, Nakai-Murakami C, Osawa Y, Tokunaga K, Sata T, *et al.* Modulation of TNF-alpha-converting enzyme by the spike protein of SARS-CoV and ACE2 induces TNF-alpha production and facilitates viral entry. *Proc Natl Acad Sci U S A* 2008; **105**:7809–7814.
28 Lambert DW, Yarski M, Warner FJ, Thornhill P, Parkin ET, Smith AI, *et al.* Tumor necrosis factor-alpha convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2). *J Biol Chem* 2005; **280**:30113–30119.
29 Qu D, Wang Y, Esmon NL, Esmon CT. Regulated endothelial protein C receptor shedding is mediated by tumor necrosis factor-alpha converting enzyme/ADAM17. *J Thromb Haemost* 2007; **5**:395–402.
30 Fukudome K, Esmon CT. Identification, cloning, and regulation of a novel endothelial cell protein C/activated protein C receptor. *J Biol Chem* 1994; **269**:26486–26491.
31 Ge N, Xia Q, Yang ZH, Ding QF, Zeng Z. Vascular endothelial injury and apoptosis in rats with severe acute pancreatitis. *Gastroenterol Res Pract* 2015; **2015**:235017.
32 Yan W, Chen D, Bigambo FM, Wei H, Wang X, Xia Y. Differences of blood cells, lymphocyte subsets and cytokines in COVID-19 patients with different clinical stages: a network meta-analysis. *BMC Infect Dis* 2021; **21**:156.
33 Pestka SB. Old drug, new trick? The rationale for the treatment of COVID-19 with activated protein C. *Med Hypotheses* 2021; **149**:110537.
34 Simpson S, Kay FU, Abbara S, Bhalla S, Chung JH, Chung M, *et al.* Radiological Society of North America Expert Consensus Statement on Reporting Chest CT Findings Related to COVID-19. Endorsed by the Society of Thoracic Radiology, the American College of Radiology, and RSNA - Secondary Publication. *J Thorac Imaging* 2020; **35**:219–227.
35 Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, *et al.* Correlation of chest CT and RT-PCR testing for coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. *Radiology* 2020; **296**:E32–E40.
36 Bao C, Liu X, Zhang H, Li Y, Liu J. Coronavirus disease 2019 (COVID-19) CT findings: a systematic review and meta-analysis. *J Am Coll Radiol* 2020; **17**:701–709.