Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Thrombosis Research 204 (2021) 13–15

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Letter to the Editors-in-Chief

Low admission protein C levels are a risk factor for disease worsening and mortality in hospitalized patients with COVID-19

ARTICLE INFO

Keywords
Anticoagulant
COVID-19
Fibrinolysis
Protein C
SARS-CoV-2

At present, the clinical course of coronavirus disease 2019 (COVID-19) is unpredictable and can rapidly develop, causing severe and deadly complications. Therefore, there is an urgent need to identify reliable biomarkers related to COVID-19 disease progression and death for diagnostics as well as to identify pathways that are amenable to existing or new therapeutics. While biomarkers of coagulation (e.g. D-dimer), inflammation (e.g. interleukin-6 [IL-6] and C-reactive protein [CRP]), cell damage (e.g. lactate dehydrogenase [LDH]) and immunity (e.g. lymphocyte count) as well as clinical scoring systems (e.g. International Society on Thrombosis and Hemostasis [ISTH] disseminated intravascular coagulation [DIC] score) [1] can be helpful in predicting clinical course and outcome in patients with COVID-19, there is a need for additional biomarkers.

As COVID-19 progresses, inflammatory responses lead to a coagulopathy associated with a high incidence of thrombotic events, especially in the microvasculature [2]. The pattern of changes in hemostatic variables in COVID-19-associated coagulopathy appears to be different to that in sepsis and DIC, and there are gaps in knowledge as to which hemostatic proteins that may be most informative for the early identification of patients with poor prognosis in COVID-19 [2].

We aimed to characterize admission plasma levels of 12 hemostatic proteins in hospitalized COVID-19 patients in order to identify proteins associated with risk of disease worsening including death within 28 days. The data used here is from a publicly available longitudinal COVID-19 cohort collected at the Massachusetts General Hospital (MGH), Boston, USA (with institutional review board approval; https://www.olink.com/mgh-covid-study/), which has recently been described in detail [3].

This study is based on 231 COVID-19 patients presenting at the emergency department with moderate or severe illness, i.e. requiring oxygen (n = 152) or intensive care (n = 79). The World Health Organization (WHO) COVID-19 outcomes scale was used on day one and again at 28-day follow-up to classify patients as mild (WHO 5–6), moderate (WHO 4), severe (WHO 2–3) or dead (WHO 1). Of the 152 patients presenting with moderate COVID-19, 128 improved, 2 remained unchanged, 5 deteriorated and 17 died; of the 79 patients with severe COVID-19, 23 improved, 34 remained unchanged, 0 deteriorated and 22 died.

Plasma was isolated from blood collected in EDTA tubes on admission. Plasma protein levels were measured using proximity extension assay (PEA) technology with the OLINK Explore 1536 panel (OLINK, Uppsala, Sweden). In total 12 proteins belonging to the Kyoto Encyclopedia of Genes and Genomes (KEGG) coagulation cascade were present on the OLINK Explore panel and analyzed for association with 28-day disease worsening in the present study.

Available clinical and admission laboratory measures previously reported to associate with COVID-19 clinical course and/or outcomes that were included as covariates in the multivariate analyses in the present study were coded as binary variables as indicated in Table 1 (for original definitions, see https://www.olink.com/mgh-covid-study/ and [3]). Given that IL-6 has been robustly associated with poor prognosis in COVID-19 patients, IL-6 levels measured with the OLINK Explore panel were also included as a covariate. Identifying variables such as sex and ethnicity were unavailable. In line with previous studies, patients whose condition deteriorated (including those who died) were more likely to be older, have comorbidities, reduced lymphocyte count, and increased creatinine, CRP, D-dimers, LDH and IL-6 than patients who improved (Table 1).

Univariate ordinal logistic regression analyses for 28-day disease worsening were performed for each of the 12 hemostatic proteins. Patients whose condition deteriorated had elevated admission plasma levels of proteinase-activated receptor 1 (PAR1, $p = 0.004$), tissue factor

Abbreviations: APC, activated protein C; DIC, disseminated intravascular coagulation; CRP, C-reactive protein; FVII, coagulation factor VII; FIX, coagulation factor IX; IL-6, interleukin-6; LDH, lactate dehydrogenase; PAI-1, plasminogen activator inhibitor type 1; PAR-1, proteinase-activated receptor 1; TF, tissue factor; TFPI, tissue factor pathway inhibitor; t-PA, tissue-type plasminogen activator; TM, thrombomodulin; u-PA, urokinase-type plasminogen activator; uPAR, urokinase-type plasminogen activator receptor; vWF, von Willebrand factor.

https://doi.org/10.1016/j.thromres.2021.05.016
Received 27 January 2021; Received in revised form 6 May 2021; Accepted 25 May 2021
Available online 29 May 2021

Table 1
Clinical variables of moderate and severe COVID-19 patients included as covariates in multivariable regression analyses, stratified based on whether patient condition improved, remained unchanged or deteriorated (including death) over 28 days.

Variables	Improved (n = 151)	Unchanged (n = 36)	Deteriorated (n = 44)	*p*-Value
Clinical variables				
Age >65y, n (%)	47 (31.1)	16 (44.5)	39 (88.6)	<0.001
BMI >30 kg/m^2, n (%)	77 (51.0)	13 (36.1)	15 (34.1)	0.066
Heart disease, n (%)	22 (14.6)	2 (5.6)	17 (38.6)	<0.001
Lung disease, n (%)	37 (24.5)	2 (5.8)	11 (25.0)	0.038
Diabetes mellitus, n (%)	51 (33.8)	22 (61.1)	18 (40.9)	0.010
Hypertension, n (%)	61 (40.4)	21 (58.3)	34 (77.3)	<0.001
Immunocompromized, n (%)	9 (6.0)	6 (16.7)	6 (13.6)	0.068
Admission laboratory measures				
Lymphocyte count <1.5 10^9/L	119 (78.8)	33 (94.3)	39 (92.9)	0.017
Creatinine ≥1.8 mg/dL	10 (6.6)	6 (16.7)	13 (31.0)	<0.001
CRP ≥100 mg/L	77 (51.0)	32 (88.9)	30 (71.4)	<0.001
D-dimer ≥1000 ng/mL	75 (51.0)	24 (66.7)	32 (76.2)	0.008
LDH ≥400 U/L	51 (34.0)	25 (69.4)	21 (50.0)	<0.001
IL-6 mean NPX (+/−SD)	5.3 (1.3)	6.9 (1.6)	6.8 (1.7)	<0.001

Differences in variables were evaluated using Pearson's χ^2 test for categorical variables and ANOVA for IL-6. BMI, body mass index; CRP, C-reactive protein; IL-6, interleukin-6; LDH, lactate dehydrogenase; n, number; NPX, Normalized Protein eXpression values; and SD, standard deviation.

(TF, $p = 2.9E{-}08$), tissue factor pathway inhibitor (TFPI, $p = 0.024$), thrombomodulin (TM, $p = 5.4E{-}6$), tissue-type plasminogen activator (t-PA, $p = 3.5E{-}6$), urokinase-type plasminogen activator receptor (uPAR, $p = 2.4E{-}7$), and von Willebrand factor (vWF, $p = 8.1E{-}5$), and lower levels of coagulation factor VII (FVII, $p = 0.002$) and protein C ($p = 1.2E{-}6$; Fig. 1A). No significant association with disease worsening was detected for coagulation factor IX (FIX, $p = 0.95$), plasminogen activator inhibitor type1 (PAI-1, $p = 0.30$), or urokinase-type plasminogen activator (u-PA, $p = 0.13$).

When each of the significant hemostatic proteins were assessed for association with disease worsening in multivariate ordinal logistic regressions when adjusting for clinical variables (age, body mass index [BMI], and comorbidities), elevated levels of five proteins (TF, TM, t-PA, uPAR, and vWF) and reduced levels of two proteins (FVII and protein C) remained significant (data not shown). When further adjusting for admission laboratory measures, only lower FVII and protein C remained significantly associated with disease worsening (Fig. 1B). In the final regression model that included both proteins, only age (odds ratio [OR] 5.0, 95% confidence interval [CI] 2.4–10.1, $p < 0.001$), IL-6 (OR 1.6, 95% CI 1.3–2.0, $p = 0.001$), creatinine (OR 3.1, 95% CI 1.4–6.6, $p = 0.017$) and protein C (OR 0.38, 95% CI 0.20–0.73, $p = 0.016$) remained significantly associated with disease worsening. It deserves mention that D-dimer was not retained in this model (OR 0.86, 95% CI 0.45–1.64, $p = 0.70$). Correlations between the investigated biomarkers were evaluated using the Pearson method. Protein C was most strongly correlated to FVII ($r = 0.6$), followed by FIX ($r = 0.5$) and TFPI ($r = 0.4$) and was inversely correlated to IL-6 ($r = -0.3$; $p < 0.001$ for all).

Given that a relatively high proportion of patients with heart conditions are treated with anticoagulants that can affect some hemostatic protein levels, we excluded 41 cases with these conditions in a sensitivity analysis (n = 29 moderate and n = 12 severe). Admission protein C levels were associated with 28-day disease worsening also in this analysis (OR 0.31, 95% CI 0.15–0.65, $p = 0.011$), indicating that oral anticoagulant use did not contribute to our initial finding.

We next evaluated the predictive value of protein C above clinical and routine admission laboratory parameters for risk of disease worsening by using area under the receiver operating characteristic curves (AUC; Fig. 1C). On their own, clinical and laboratory measures had good discriminating capacity to identify patients whose condition deteriorated and/or who died compared to improved (AUC 0.90, 95% CI 0.85–0.95). When adding protein C levels, the AUC increased modestly, but significantly ($p < 0.001$), to 0.92 (95% CI 0.88–0.96).

Protein C is located on the surface of endothelial cells (EC). Together with protein S, activated protein C (APC) is a strong inhibitor of the coagulation system, especially in the microcirculation. APC also reduces inflammation, apoptosis and stabilizes endothelial and epithelial barriers [4,5]. Thus, APC is involved in many of the pathologic changes that occur in COVID-19. Depleted protein C (measured by chromogenic assays and/or ELISA) has been associated with DIC and poor prognosis in sepsis [5]. In a recent study, low protein C levels measured by ELISA were one of several variables that could identify the pre-DIC state in septic patients [6]. Of note, protein C and APC have short half-lives and situations with increased activation of the coagulation pathway thus result in acquired deficiency of protein C and the development of a thrombogenic state [7]. Therefore, the reduction in admission protein C levels observed here is likely attributable to increased consumption and indicative of DIC rather than to reduced synthesis in the liver.

In regards to previous studies on protein C in patients with COVID-19, one study of consecutive patients with varying degrees of illness found that 7% had protein C activity levels below the normal reference range [8], and two studies that included only patients requiring intensive care (defined here as severe) reported low protein C activity levels in patients [9,10]. However, conflicting data was also found in a small study of 11 severely ill COVID-19 patients [11]. In terms of associations to disease severity, one study of 30 patients requiring intensive care

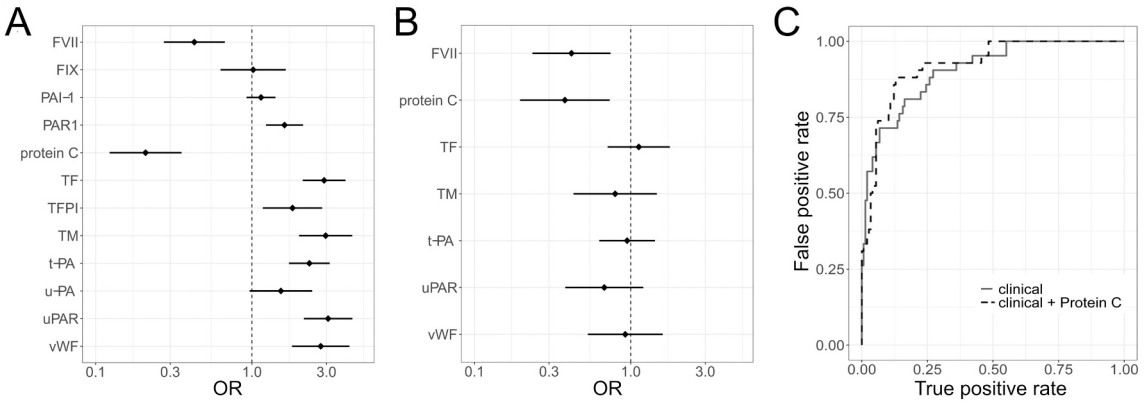

Fig. 1. Plasma levels of hemostatic proteins and 28-day disease worsening in hospitalized COVID-19 patients. Odds ratio (OR) and 95% confidence intervals for disease worsening from (A) univariate ordinal logistic regression and (B) multivariate ordinal logistic regression (adjusted for clinical variables and admission laboratory measures; see Table 1). (C): Receiver operating characteristic curves showing the predictive value of protein C over clinical variables and laboratory parameters.

found patients with a high sequential organ failure assessment (SOFA) score to have lower protein C activity compared to those with a low SOFA score [12]. Finally, patients requiring intensive care had significantly lower protein C activity than patients treated at the regular hospital ward (defined here as mild or moderate) in the COMPASS-COVID-19 study [1]. The novel finding in the present study is that low hospital admission levels of protein C (i.e. prior to any prophylactic treatment or intubation) in patients presenting with moderate or severe COVID-19 are independently associated with 28-day disease worsening and mortality. This study is based on PEA technology and reflects the total circulating levels of protein C. Going forward, it will be crucial to replicate these results using clinically relevant protein C assays (e.g. chromogenic and/or clotting based activity assays).

Given the fact that thrombosis can occur despite prophylactic and therapeutic use of heparin, and that bleeding is rare in COVID-19 patients even in the setting of thrombocytopenia and DIC [13], additional pharmaceutical interventions may be beneficial in some individuals. In line with this, it has been suggested that drugs modulating the protein C pathway merit exploration in the treatment of critically ill COVID-19 patients [4].

To conclude, here we provide evidence that lowered hospital admission plasma levels of protein C in COVID-19 patients presenting with moderate or severe illness may be a useful biomarker for disease worsening and high mortality risk. Further studies using clinically relevant protein C assays (e.g. activity assays) are needed in order to replicate this finding. An important next step will be to evaluate whether admission protein C activity levels can predict thromboembolic complications.

Funding sources

This work was supported by the Swedish Research Council (2018-02543); the Swedish Heart-Lung Foundation (20190203); the Swedish state, under an agreement between the Swedish government and the county councils (ALF agreement ALFGBG-720081 and ALFGBG-717531); and by SciLifeLab Sweden (KAW 2020.0182).

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

T.M. Stanne, A. Pedersen and C. Jern have nothing to disclose. M Gisslen reports personal fees from Amgen, Biogen, Gilead, GSK/ViiV, Novocure and Novo Nordic all of which are outside the scope of the submitted work.

Acknowledgement

Data was provided by the MGH Emergency Department COVID-19 Cohort (Filbin, Goldberg, Hacohen) with Olink Proteomics. We would like to thank MD Cecilia Lagging, MSc Sofia Klasson, and PhD Björn Andersson for discussion and statistical support.

References

[1] G.T. Gerotziafas, T.N. Sergentanis, G. Voiriot, L. Lassel, C. Papageorgiou, A. Elabbadi, M. Turpin, P. Vandreden, L. Papageorgiou, T. Psaltopoulou, E. Terpos, M.A. Dimopoulos, A. Parrot, J. Cadranel, G. Pialoux, M. Fartoukh, I. Elalamy, Derivation and validation of a predictive score for disease worsening in patients with COVID-19, Thromb. Haemost. 120 (2020) 1680–1690, https://doi.org/10.1055/s-0040-1716544.

[2] M. Levi, J. Thachil, Coronavirus disease 2019 coagulopathy: disseminated intravascular coagulation and thrombotic microangiopathy—either, neither, or both, Semin. Thromb. Hemost. 46 (2020) 781–784, https://doi.org/10.1055/s-0040-1712156.

[3] Filbin MR, Mehta A, Schneider AM, Kays KR, Guess JR, Gentili M, Fenyves BG, Charland NC, Gonye ALK, Gushterova I, Khanna HK, LaSalle TJ, Lavin-Parsons KM, Lilly BM, Lodenstein CL, Manakongtreecheep K, Margolin JD, McKaig BN, Rojas-Lopez M, Russo BC, et al. Plasma proteomics reveals tissue-specific cell death and mediators of cell-cell interactions in severe COVID-19 patients. bioRxiv. 2020; 2020.11.02.365536. doi:https://doi.org/10.1101/2020.11.02.365536. Preprint.

[4] M. Mazzeffi, J.H. Chow, A. Amoroso, K. Tanaka, Revisiting the protein C pathway: an opportunity for adjunctive intervention in COVID-19? Anesth. Analg. 131 (2020) 690–693, https://doi.org/10.1213/ANE.0000000000005059.

[5] S.N. Faust, M. Levin, O.B. Harrison, R.D. Goldin, M.S. Lockhart, S. Kondaveeti, Z. Laszik, C.T. Esmon, R.S. Heyderman, Dysfunction of endothelial protein C activation in severe meningococcal sepsis, N. Engl. J. Med. 345 (2001) 408–416, https://doi.org/10.1056/NEJM200108093450603.

[6] N.L. Jackson Chornenki, D.J. Dwivedi, A.C. Kwong, N. Zamir, A.E. Fox-Robichaud, Liaw PC; Canadian Critical Care Translational Biology Group. Identification of hemostatic markers that define the pre-DIC state: a multi-center observational study, J. Thromb. Haemost. 18 (2020) 2524–2531, https://doi.org/10.1111/jth.14973.

[7] P.N. Knoebl, Severe congenital protein C deficiency: the use of protein C concentrates (human) as replacement therapy for life-threatening blood-clotting complications, Biologics. 2 (2008) 285–296, https://doi.org/10.2147/btt.s1954.

[8] M.T. Calderon-Lopez, N. Garcia-Leon, S. Gomez-Arevalillo, P. Martin-Serrano, A. Matilla-Garcia, Coronavirus disease 2019 and coagulopathy: other prothrombotic coagulation factors, Blood Coagul. Fibrinolysis 32 (2021) 44–49, https://doi.org/10.1097/MBC.0000000000000996.

[9] Y. Zhang, W. Cao, W. Jiang, M. Xiao, Y. Li, N. Tang, Z. Liu, X. Yan, Y. Zhao, T. Li, T. Zhu, Profile of natural anticoagulant, coagulant factor and anti-phospholipid antibody in critically ill COVID-19 patients, J. Thromb. Thrombolysis 50 (2020) 580–586, https://doi.org/10.1007/s11239-020-02182-9.

[10] A. Tabatabai, J. Rabin, J. Menaker, R. Madathil, S. Galvagno, A. Menne, J.H. Chow, A. Grazioli, D. Herr, K. Tanaka, T. Scalea, M. Mazzeffi, Factor VIII and functional protein C activity in critically ill patients with coronavirus disease 2019: a case series, A A Pract. 14 (2020), e01236, https://doi.org/10.1213/XAA.0000000000001236.

[11] M. Panigada, N. Bottino, P. Tagliabue, G. Grasselli, C. Novembrino, V. Chantarangkul, A. Pesenti, F. Peyvandi, A. Tripodi, Hypercoagulability of COVID-19 patients in intensive care unit: a report of thromboelastography findings and other parameters of hemostasis, J. Thromb. Haemost. 18 (2020) 1738–1742, https://doi.org/10.1111/jth.14850.

[12] T.D. Corrêa, R.L. Cordioli, J.C. Campos Guerra, B. Caldin da Silva, R. Dos Reis Rodrigues, G.M. de Souza, T.D. Midega, N.S. Campos, B.V. Carneiro, Campos FND, H.P. Guimarães, G.F.J. de Matos, V.F. de Aranda, L.J. Rolim Ferraz, Coagulation profile of COVID-19 patients admitted to the ICU: an exploratory study, PLoS One 15 (2020), e0243604, https://doi.org/10.1371/journal.pone.0243604.

[13] J.M. Connors, J.H. Levy, COVID-19 and its implications for thrombosis and anticoagulation, Blood. 135 (2020) 2033–2040, https://doi.org/10.1182/blood.2020006000.

Tara M. Stanne[a,*], Annie Pedersen[a,b], Magnus Gisslén[c,d], Christina Jern[a,b]

[a] *Institute of Biomedicine, Department of Laboratory Medicine, the Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden*

[b] *Region Västra Götaland, Sahlgrenska University Hospital, Department of Clinical Genetics and Genomics, Gothenburg, Sweden*

[c] *Institute of Biomedicine, Department of Infectious Diseases, the Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden*

[d] *Region Västra Götaland, Sahlgrenska University Hospital, Department of Infectious Diseases, Gothenburg, Sweden*

[*] Corresponding author at: Institute of Biomedicine, Department of Laboratory Medicine, the Sahlgrenska Academy, University of Gothenburg, SE-40530 Gothenburg, Sweden.
E-mail address: tara.stanne@gu.se (T.M. Stanne).