RESEARCH LETTER

Relationship between COVID-19 severity, markers of endothelial impairment, and Simple Covid Risk Index

Mateusz Fabiś[1*], Paulina Gorzelak-Pabiś[1*], Joanna Satała[1], Agnieszka Pawlos[1], Jarosław Fabiś[2], Marlena Broncel[1]

1 Department of Internal Diseases and Clinical Pharmacology, Laboratory of Tissue Immunopharmacology, Medical University of Lodz, Łódź, Poland
2 Department of Arthroscopy, Minimally Invasive Surgery and Sports Traumatology, Medical University of Lodz, Łódź, Poland

Correspondence to:
Paulina Gorzelak-Pabiś, MD, PhD, Deptartment of Internal Diseases and Clinical Pharmacology, Laboratory of Tissue Immunopharmacology, Medical University of Lodz, ul. Kniaziewicza 1/5, 91-347 Łódź, Poland, phone: +48 42 251 60 03, email: paulina.gorzelak-pabis@umed.lodz.pl
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* MF and PGP contributed equally to this work.

Introduction Recent evidence suggests that vascular endothelial cells may play a crucial role in COVID-19 development. SARS-CoV-2 enters human cells through the angiotensin-converting enzyme-2 receptor,[1] a protein abundantly expressed in endothelial cells, and causes endothelial dysfunction, apoptosis, and necrosis.[2] The lungs have the highest amount of endothelial cells and their damage may lead to acute respiratory distress syndrome (ARDS). Furthermore, the endothelium mediates cell communication between the immune and coagulation systems in response to infection. This process is known as immunothrombosis and refers to clot formation initiated by the immune system.[3,4] SARS-CoV-2 infection induces both immunological and thrombotic dysregulation.[2] There are indications suggesting that the interaction of von Willebrand factor (VWF) with platelets is most efficient under low flow conditions observed in immunothrombosis.[5] As a result, thrombotic complications may affect multiple organs beside the lungs and lead to clinical deterioration. This disseminated microthrombosis is manifested by increased D-dimer levels.[6] Lymphopenia is a key pathophysiological feature of COVID-19.[7] Numerous studies have demonstrated a correlation between lymphopenia and the disease severity,[8] however, the exact mechanism underlying the observed lymphopenia is still a matter of debate.

In our study, we have attempted to create a simple prognostic index of COVID-19 severity, considering both immunological and thrombotic factors based on lymphocyte count and D-dimer concentration. We proposed the Simple Covid Risk Index (SCRI) (lymphocyte / D-dimer ratio) as a new tool for predicting the course of COVID-19 on the day of admission to a hospital.

Patients and methods The study included 80 adult patients with laboratory-confirmed SARS-CoV-2 infection. Several parameters were recorded on admission, including age and sex, and routine blood samples were collected. Lymphocytes, D-dimer, and serum C-reactive protein (CRP) and interleukin 6 (IL-6) were determined by conventional laboratory methods. The concentrations of vascular endothelial growth factor (VEGF), vascular cell adhesion protein 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1) in the serum and tissue factor (TF) concentration in the plasma were determined by the sandwich enzyme-linked immunosorbent assay according to the manufacturer's (Cloud-Clone Corp., Katy, Texas, United States) instructions. The patients were divided into 2 groups with moderate and severe course of COVID-19. The patients with lung computed tomography (CT) images showing a moderate or minimal progress in the lesion size below 25% and meeting 2 of the following criteria: 1) oxygen saturation above 91% at rest; 2) CRP below 40 mg/l; and 3) IL-6 below 30 mg/l were categorized as a group with moderate course of COVID-19. The patients with lung CT images showing clear and extensive progress of the lesion size equal to or greater than 25% and meeting 2 of the following criteria: 1) oxygen saturation equal to or below 91% at rest; 2) CRP equal to or above 40 mg/l; and 3) IL-6 equal to or above 30 mg/l were categorized as a group with severe course of COVID-19. The primary objective of our study was to assess the prognostic value of selected factors on the course of SARS-CoV-2 infection. To this end, we measured serum and plasma levels of essential endothelial molecules, such as ICAM-1, VWF,

VCAM-1, VEGF, and TF, and compared their concentrations in patients with severe and moderate course of COVID-19. The secondary objective of the study was to assess the potential prognostic value of the SCRI (lymphocyte [10^3/μl] / D-dimer [μg FEU/l] ratio) on the first day of hospitalization.

Statistical analysis The relationships between pairs of groups were tested with the *t* test (normal distribution) and the Mann–Whitney test (non-normal distribution). Multivariable logistic regression was also generated, using severe COVID-19 course as an outcome and the selected variables including D-dimers, sex, age, ICAM-1, VEGF, TF, VCAM-1, lymphocyte / D-dimer ratio, and VWF as predictors. The predictive power was evaluated by receiver operating characteristics (ROC) and area under the curve (AUC). The optimal cutoff point in the ROC analysis was chosen with the use of an online tool 'CutoffFinder' (http://molpath.charite.de/cutoff) using 'Manhattan distance' (Institut für Pathologie, Charité-Universitätsmedizin Berlin, Berlin, Germany.[9] Statistical analyses were performed with the GraphPad Prism 9.0 (GraphPad Software, San Diego, California, United States) and Statistica software (StatSoft, Inc., Kraków, Poland). All tests were considered significant at *P* value below 0.05. The investigation was approved by the Bioethics Committee of the Medical University of Lodz (RNN/122/21/KE).

Results Of the 80 enrolled patients 43 (54%) were men and 37 (46%) were women. The mean (SD) age was 64 (14) years. For further analysis, the patients were divided into 2 groups of moderate COVID-19 course and severe COVID-19 course, according to the study design. The median values and differences between the patients with moderate and severe COVID-19 are shown in Supplementary material, *Table S1*.

Severe course of COVID-19 was associated with more extensive endothelial damage and higher activity of the markers than moderate course of the infection. The 42 patients with severe disease had the following median (interquartile range [IQR]) levels of the investigated markers: ICAM-1, 178.4 (157.8–221.0) ng/ml; VCAM-1, 750.7 (482.2–1245) ng/ml; VWF, 253.5% (208.5–420.8); TF, 564.2 (197.6–923.2) pg/ml; and VEGF, 13 141 (8261–15 331) pq/ml. In the 38 patients with moderate disease the median (IQR) values of the markers reached 153.9 (134.5–188.9) ng/ml for ICAM-1, 399.4 (267.0–794.2) ng/ml for VCAM-1, 216.5 (191.8–319.8)% for VWF, 275.9 (95.9–663.2) pg/ml for TF, and 8746 (4803–12 384) pq/ml for VEGF (FIGURE 1A–1E).

The patients with severe course of COVID-19 had significantly lower SCRI on admission than those with moderate disease (FIGURE 1F). We plotted the ROC of SCRI for predicting severe course of COVID-19 (AUC 0.83, $P < 0.001$), and the optimal cutoff point with sensitivity (95.24%) and specificity (65.79%) was 1.17 (FIGURE 1G).

ICAM-1 and SCRI showed significant prognostic value for COVID-19 course in the multiple logistic regression model. They were the only factors predicting the severity of the disease that were independent of other parameters included in the analysis (FIGURE 1H). An increase in ICAM-1 concentration by 25, 50, 100, and 150 ng/ml enhanced the chance for the severe course of COVID-19 by 2.04, 4.16, 17.29, and 71.88 times, respectively, if other parameters remained unchanged.

Discussion The first finding of our study was that the patients with severe COVID-19 had significantly higher concentrations of key markers associated with endothelial damage and endothelial activity, including ICAM-1, VCAM-1, VEGF, VWF, and TF, in blood plasma / serum than the patients experiencing moderate clinical symptoms. Previous reports have confirmed the elevation of these factors, however, no study before has simultaneously considered all the parameters we examined. Also, there are no studies comparing the levels of these parameters between severe and moderate course of COVID-19 in patients not requiring mechanical ventilation on admission.

ICAM-1 and VCAM-1 control transmigration of leukocytes from blood vessels through the vascular endothelium toward inflammation in the tissues. The key role of ICAM-1 is binding leukocytes to the endothelial cells and activating leukocyte transmigration.[10] VCAM-1 mediates the adhesion of leukocytes, such as T lymphocytes and macrophages, to vascular endothelium.[11] In our study, median serum levels of ICAM-1 and VCAM-1 in the patients with severe COVID-19 were higher by 16% and 88%, respectively, than in the group with moderate course of the disease. According to previous studies, VEGF mediates pathogenesis of ARDS and acute lung injury by increasing vascular permeability. VEGF increases microvascular permeability 20 000 times more potently than histamine.[12] The increase in vascular permeability promotes leukocyte transmigration.[5] We found that the patients with severe COVID-19 had by 50% higher median VEGF serum concentrations than those with moderate COVID-19. Furthermore, VEGF may promote coagulation, since it increases the expression of TF in endothelial cells.[13] TF is the primary initiator of the blood coagulation cascade. Together with factor VIIa, they form a complex that catalyzes the conversion of factor X into active factor Xa.[14] Endothelial cells and monocytes / macrophages release TF in response to inflammatory mediators.[11] Another essential hemostatic molecule is VWF, an adhesion glycoprotein of plasma that has multiple functions. VWF binds to factor VIII, platelet surface glycoproteins, and collagen. VWF is synthesized and stored by vascular endothelial cells.[15] The patients with severe COVID-19 had significantly higher median serum VWF and plasma TF levels, by 17% and 105%, respectively, than those with moderate COVID-19.

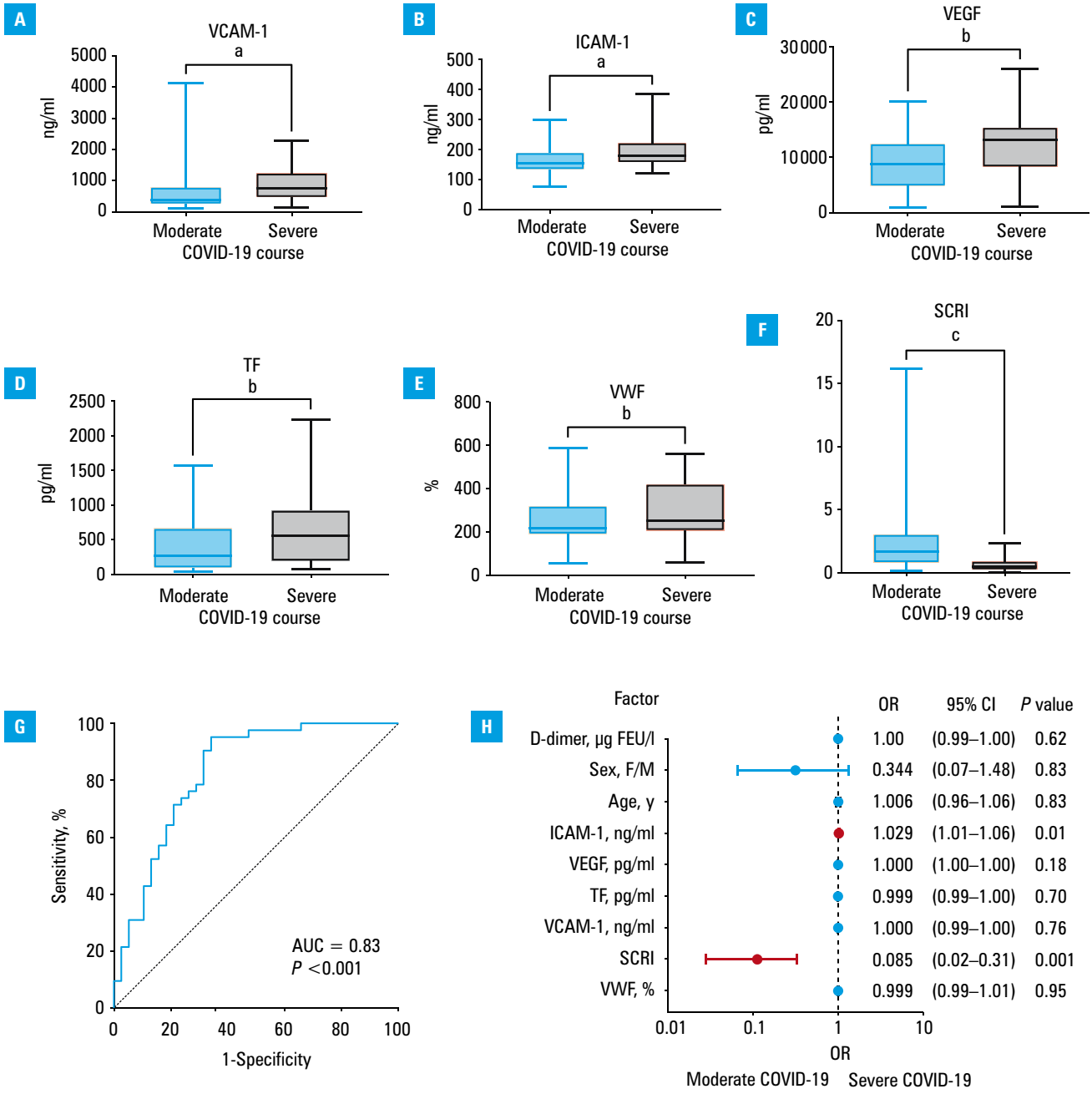

FIGURE 1 Markers associated with endothelial impairment in patients with severe and moderate COVID-19 course (boxes – medians with interquartile ranges [IQRs], whiskers – minimal and maximal value, bars – mean with SD); **A** – $P = 0.001$; **B** – $P = 0.004$; **C** – $P = 0.02$; **D** – $P = 0.02$; **E** – $P = 0.04$; **F** – $P < 0.001$; lymphocyte/D-dimer ratio on admission in patients with moderate and severe course of COVID-19; **G** – ROC curve of lymphocyte/D-dimer ratio (SCRI); **H** – regression model for predicting the severe course of COVID-19 (multivariable logistic regression);
a $P < 0.01$, **b** $P < 0.05$, **c** $P < 0.0001$
Abbreviations: AUC, area under the curve; F, female; ICAM-1, intercellular adhesion molecule 1; M, male; OR, odds ratio; ROC, receiver operating characteristics; SCRI, Simple Covid Risk Index; TF, tissue factor; VCAM-1, vascular cell adhesion protein 1; VEGF, vascular endothelial growth factor; VWF, von Willebrand factor

The second finding of our study, which seems even more relevant and useful in clinical practice, is noticing the possible predictive value of the lymphocyte to D-dimer ratio (SCRI). SCRI is associated with the severity of clinical course of the disease and can be calculated on the first day of hospitalization. The index has high sensitivity, and a decent specificity value. What is particularly interesting is that the ratio of lymphocytes to D-dimers is better at predicting the course of COVID-19 than the level of D-dimers alone.

Conclusions We showed that the patients with severe COVID-19 had significantly higher levels of immune and thrombotic endothelial activation factors than those with moderate disease symptoms. High sensitivity and specificity of SCRI suggests that it may be useful in everyday clinical practice to predict whether COVID-19 patients would develop moderate or severe disease.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/paim.

ARTICLE INFORMATION

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CONFLICT OF INTEREST None declared.

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