Estimated plasma volume status in COVID-19 patients and its relation to comorbidities and clinical outcomes

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Abstract:

Background and Aims: Blood plasma is a large reservoir of circulating mediators of inflammation and its expansion has been associated with unfavorable outcomes in patients with inflammatory and cardiovascular diseases. The aim of this study was to determine clinical and prognostic value of estimated plasma volume status (ePVS) in hospitalized patients with COVID-19.

Methods: We retrospectively investigated 5871 consecutive COVID-19 patient hospitalized in our tertiary-level institution in period 3/2020-6/2021. ePVS was determined using the Strauss-derived Duarte formula and was correlated with clinical characteristics and unwanted outcomes.

Results: Median ePVS was 4.77 dl/g with interquartile range 4.11-5.74. Higher ePVS was significantly associated with older age, female sex, higher comorbidity burden, worse functional status, less severe COVID-19 clinical presentation with lower severity and longer duration of symptoms, but more pronounced inflammatory profile with higher C-reactive protein, interleukin-6 and D-dimer levels ($P<0.05$ for all analyses). In the multivariate regression analysis U shaped relationship of ePVS with mortality was revealed, present independently of age, sex, COVID-19 severity and comorbidity burden. In addition, higher ePVS was independently associated with higher tendency for mechanical ventilation, intensive care unit treatment, venous thromboembolism, major bleeding and bacteriemia and lower ePVS was independently associated with tendency for arterial thrombotic events.

Conclusion: Higher ePVS, indicative of plasma volume expansion and inflammatory cytokine accumulation, may predispose respiratory deterioration and venous thromboembolism, despite less severe initial clinical presentation. Lower ePVS, indicative of hemoconcentration, may predispose arterial thrombotic events. Both may be associated with higher mortality in hospitalized COVID-19 patients.

Keywords: blood plasma; COVID-19; hemoconcentration; volume overload; prognosis

Introduction:

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a systemic inflammatory disease presenting dominantly with respiratory symptoms. Although majority of infected patients experience asymptomatic disease or only mild symptoms, prior to availability of vaccination up to 15-20% of patients developed respiratory insufficiency and systemic inflammatory response, requiring hospitalization [1]. Tendency for rapid respiratory deterioration and thrombotic complications [2] are striking features of severe COVID-19, associated with underlying thromboinflammation specific for the disease [3]. Age, male sex and comorbidities, especially chronic metabolic diseases with cardiovascular complications, are known predisposing factors for unfavorable clinical outcomes in COVID-19 patients [4]. Vaccine hesitancy, waning effects of vaccination and inappropriate immunization in immunosuppressed patients still make severe forms of the disease a constant threat despite occurrence of novel therapeutic options and less aggressive viral strains.

Blood plasma, non-cellular component of the blood, constitutes more than half of circulating blood volume. It is a large reservoir of cytokines, other mediators of inflammation, hormones, nutrients and metabolites of various metabolic processes. Increased estimated plasma volume status (ePVS) has been associated with increased thrombotic and mortality risk in the general population [5] and in cohorts of patients with established cardiovascular [6-8] and inflammatory diseases [9, 10]. Nevertheless, its role in patients with severe and critical COVID-19 has not been evaluated so far. The aim of this study was to determine clinical and prognostic value of estimated plasma volume status (ePVS) in hospitalized patients with COVID-19.

Methods:

We retrospectively investigated a cohort of 5871 consecutive hospitalized COVID-19 patients treated in our tertiary center institution, University hospital Dubrava in period 3/2020-6/2021 who had available data on hematocrit and hemoglobin at the time of hospital admission. During this

period the institution was completely repurposed to serve as a COVID-19 regional referral center for most severe clinical presentations and for patients with acute medical, surgical or neurological emergencies that were concomitantly SARS-CoV-2 positive. All patients were adults and of White race. All patients tested positive on either PCR or antigen test in the presence of compatible clinical presentation. Patients were treated according to the contemporary guidelines with majority receiving low molecular weight heparin and corticosteroids of various dose intensity and duration. Data on demographic and clinical characteristics, comorbidities, laboratory parameters and the course of hospitalization were obtained through analysis of electronical and written medical documentation and are a part of a hospital registry project. Outcomes of death during hospitalization, requirement for mechanical ventilation, intensive care unit (ICU) treatment, occurrence of arterial thromboses, venous thromboembolism (VTE), major bleeding and bacteriemia were investigated. Charlson comorbidity index was used as a cumulative measure of comorbidity burden [11], in addition to evaluation of comorbidities as individual entities. The modified early warning score (MEWS) was used to as a cumulative measure of intensity of COVID-19 symptoms at the time of clinical presentation [12]. COVID-19 severity on admission was classified as mild, moderate, severe or critical based on the recommendations by the World Health Organization [13]. Complete blood count was obtained using the Advia 2120i counter (Siemens-Medical-Solutions-Diagnostics-Pte-Ltd., Swords, Ireland). ePVS was calculated using the Strauss derived Duarte formula [7]: 100-hematocrit (%)/hemoglobin (g/dL) and expressed as dl/g.

The study was approved by the University hospital Review board (nm: 2022/0706-10).

Statistical methods:

Normality of distribution of numerical variables was tested using the Kolmogorov-Smirnov test. Due to non-normal distribution in all assessed variables, they were presented as median and interquartile range (IQR) and were compared between groups using the Mann Whitney U test and

Kruskal-Wallis ANOVA test. Categorical variables were presented as frequencies and percentages and were compared between groups using the chi-squared test and chi-squared test for trend. ePVS was assessed both as a continuous variable, and as a categorical variable stratified into quartiles to assess relationship with clinical outcomes. Multivariate logistic regression analysis was used to investigate ePVS relationship with clinical outcomes after adjustments for clinically relevant parameters (age, sex, COVID-19 severity, and Charlson comorbidity index). P values <0.05 were considered as statistically significant. All analyses were performed using the MedCalc statistical software version 20.114 (MedCalc Software Ltd, Ostend, Belgium).

Results:

Overview of the patient cohort

Among a total of 5871 patients, there were 3297 (56.2%) males. Median age was 72 years, IQR (62-81). Median Charlson comorbidity index was 4 points, IQR (3-6). At the time of hospital admission, a total of 527 (9%) patients had mild, 281 (4.8%) moderate, 4166 (71%) severe, and 897 (15.3%) critical intensity of COVID-19 symptoms. A total of 1032 (17.6%) patients required mechanical ventilation, 1346 (22.9%) intensive care unit treatment, 325 (5.5%) experienced arterial and 362 (6.2%) venous thrombotic event, 184 (3.1%) had major bleeding and 608 (10.4%) had bacteriemia. A total of 1987 (33.8%) patients died.

Median hematocrit was 39%, IQR (35-42), median hemoglobin concentration was 128 g/L, IQR (114-141), and median ePVS was 4.77 dl/g, IQR (4.11-5.74).

ePVS associations demographic and clinical characteristics and prior comorbidities

Higher ePVS was statistically significantly associated with older age (median ePVS 4.92 vs 4.62 dl/g in patients ≥72 and <72 years old, P<0.001), female sex (median ePVS 5.12 vs 4.51 dl/g in female and male patients, P<0.001), higher Charlson comorbidity index (median ePVS 5.0 vs

4.51 in patients with ≥4 and <4 points, P<0.001), and worse ECOG functional status at admission (median ePVS 5 vs 4.59 dl/g in patients with ECOG ≥3 and <3 points, P<0.001).

The relationship between ePVS and particular comorbidities is shown in Table 1. Arterial hypertension, diabetes mellitus, hyperlipoproteinemia, chronic kidney disease, chronic heart failure, coronary disease, peripheral arterial disease, gastroesophageal reflux disease (GERD) and gastric ulcer, inflammatory bowel disease, chronic liver disease, liver cirrhosis, history of deep vein thrombosis, history of stroke, epilepsy, dementia, active malignancy, thyroid disease, autoimmune or rheumatic disease, and chronic hemodialysis were all statistically significantly associated with higher ePVS (P<0.05 for all comparisons). Obesity was statistically significantly associated with lower ePVS values (P<0.001).

Patients presenting with more severe intensity of COVID-19 symptoms at hospital admission had statistically significantly lower ePVS values (median 5.16, 4.88, 4.72, and 4.69 dl/g in patients with mild, moderate, severe, and critical COVID-19, respectively, P<0.001). Higher ePVS was statistically significantly associated with a longer duration of COVID-19 symptoms (median ePVS 4.58 vs 5.04 dl/g in patients with <6 and ≥6 days from symptom onset to hospitalization, P<0.001) and lower cumulative intensity of COVID-19 symptoms measured through MEWS score (median ePVS 4.96 vs 4.68 dl/g in patients with MEWS score <2 and ≥2 points, P<0.001). On the contrary, higher ePVS values were statistically significantly associated with higher CRP, higher IL-6, higher D-dimer, higher platelet count and lower ferritin, hemoglobin and WBC (P<0.05 for all analyses), as shown in Figure 1A and 1B.

ePVS associations with clinical outcomes during hospitalization

Univariate associations of ePVS stratified at quartiles with clinical outcomes are shown in Figure 2. Higher ePVS was statistically significantly associated with higher mortality during hospitalization, higher VTE and higher major bleeding rates (P<0.05 for all comparisons).

Regarding the subtype of venous thrombosis, higher ePVS was statistically significantly associated with higher rates of deep venous thrombosis (P=0.001), while there was no statistically significant difference in rates of pulmonary embolism (P=0.926). There was no statistically significant association between ePVS and the need for mechanical ventilation, treatment in the ICU, occurrence of arterial thrombosis, and bacteriemia in unadjusted analyses (P>0.05 for all analyses).

We further investigated associations of higher ePVS stratified at quartiles in a series of multivariate logistic regression models adjusted for clinically relevant variables (age, sex, presence of severe or critical COVID-19 symptoms at admission, and Charlson comorbidity index). Models are shown in Table 2. Regarding in-hospital mortality, patients belonging to the third quartile of ePVS had significantly better survival, while those belonging to the fourth quartile had worse survival compared to the first quartile, independently of age, sex, COVID-19 severity at admission, and comorbidity burden. This suggests a U-shaped association between ePVS and mortality (patients with high and low values have higher mortality). Regarding the need for mechanical ventilation, patients belonging to the third and fourth quartiles of ePVS had a significantly higher risk for mechanical ventilation compared to the first quartile, independently of age, sex, and COVID-19 severity at admission. This suggests that patients with higher ePVS had a worse clinical course of the disease with more respiratory deterioration, which was not evident in unadjusted analyses, likely due to the ePVS association with a milder COVID-19 severity at admission. Regarding the need for ICU treatment, patients belonging to the third and fourth quartiles of ePVS had a significantly higher risk for intensive care independently of age, sex, and COVID-19 severity at admission. This is in line with observations related to the need for mechanical ventilation. Regarding arterial thrombotic events, belonging to the fourth quartile of ePVS was protective against the development of arterial thrombosis, independently of COVID-19 severity at admission and comorbidity burden. Regarding VTE, patients belonging to the fourth

quartile of ePVS had a higher risk of developing VTE, independently of COVID-19 severity at admission and comorbidity burden. Regarding major bleeding, patients belonging to the fourth quartile of ePVS had a higher risk of developing major bleeding, independently of sex and COVID-19 severity at admission. Regarding the occurrence of bacteriemia, patients belonging to the fourth quartile of ePVS had a higher risk of developing bacteremia, independently of age, sex, and COVID-19 severity at admission.

Discussion:

To the best of our knowledge, out study is the first to investigate ePVS in a large cohort of real-life hospitalized COVID-19 patients with mostly severe and critical disease presentation. There are several important points we would like to emphasize.

Despite patients with higher ePVS at baseline having lower intensity of COVID-19 symptoms at the time of hospital admission, they had more pronounced inflammatory profile with higher C-reactive protein, interleukin-6 and D-dimer levels and tendency for respiratory deterioration and development of complications. Thus, despite misleadingly milder clinical presentation, they were predisposed for unfavorable course of COVID-19 infections. Dilutional effects of expanded plasma volume, but higher total inflammatory burden may result in postponed development of complications in these patients. Presence and decompensation of chronic comorbidities associated with higher ePVS may also contribute to these observations.

The U-shaped relationship of ePVS with mortality implies that both hemoconcentration and plasma volume expansion may negatively affect prognosis. Patients with higher ePVS had higher inflammatory status and more pronounced functional impairment. They were prone to progression of the COVID-19 disease itself, with propensity for respiratory deterioration, need for mechanical ventilation, ICU treatment, VTE, major bleeding and bacterial superinfections. Patients with lower ePVS had higher occurrence of arterial thrombotic events, which is possibly due to hemoconcentration and increased blood viscosity. Mechanisms underlying venous and arterial

thrombotic events in COVID-19 are incompletely understood but may differ in relationship to concomitantly observed severity of inflammation [2]. Unusually high rates of asymptomatic or oligosymptomatic VTE in severe COVID-19 patients may be observed if screening methods are utilized [14, 15], and VTE is usually associated with more severe disease presentation, ICU-level of care, more pronounced inflammation, immobilization, known inherited or acquired thrombophilia state, etc [2]. On the opposite, COVID-19 related arterial thrombotic events seem to be associated with presence of previously known atherosclerosis and less severe intensity of symptoms [2]. Inflammation, cytokine storm and coagulation are closely related in severe and critical COVID-19 patients. A number of inflammatory cytokines associated with COVID-19 activates blood and endothelial cells, inducing their procoagulant phenotype, in addition to promoting activity of coagulation cascade [16-18]. Contributing and protective effects of specific therapies used for the treatment of COVID-19 patients, including corticosteroids and LMWH in various intensities of doses, baricitinib, remdesivir [19-21] etc. are hard to decipher when evaluating retrospective cohorts of real-life patients, whereas evidence from the randomized controlled studies is scarce. This is due to a high number of confounding factors considering comorbidities, drug-drug-interactions, volumes of distribution, simultaneously protective effects due to control of inflammation and intrinsically procoagulant properties of specific medications, etc. The plasma volume may affect both the levels of circulating residual inflammation, as well as pharmacokinetics and pharmacodynamics of specific drugs. As our data show, both ePVS expansion and reduction might have prothrombotic associations in COVID-19 patients.

Age, sex, and comorbidity burden significantly affected ePVS in our study, in line with previous reports of higher ePVS in patients with cardiovascular diseases [5, 6, 22]. Large number of particular comorbidities were associated with higher ePVS in our study, including chronic heart failure, chronic kidney disease and coronary and peripheral arterial disease, and diabetes mellitus, hyperlipoproteinemia, anamnesis of CVI and VTE, chronic liver disease and liver cirrhosis, which are conditions associated with concomitant cardiovascular comorbidities or with

known expansion of plasma volume. Nevertheless, as we demonstrate, GERD/gastroesophageal ulcer disease, inflammatory bowel disease, epilepsy, dementia, active malignancy, thyroid and autoimmune/rheumatic disease were also associated with higher ePVS. These associations may be in part due to association of particular comorbidities with anemia, but also due to imbalance in body fluid regulation due to overactivation of renin-angiotensin-aldosterone neurohormonal axis [8]. Also, inflammatory drive associated with particular diseases, as well as with COVID-19 itself, may promote capillary permeability and favor plasma extravasation [23]. Obesity was associated with lower ePVS which might seem counterintuitive due to its association with other metabolic comorbidities but is in line with previous reports. Namely, obesity seems to be associated with higher augmented blood volume due to augmented body size but not in the same proportion as seen in non-obese patients and blood plasma volume per unit of body weight seems to be lower in obese than non-obese patients [24-26]. Age and comorbidity burden are known to be negative predictors of survival in hospitalized COVID-19 patients, with effects of age being moderated by sex of patients (female patients experiencing worse survival if younger, and improved survival if older in comparison to male counterparts) [4]. Are these sex-related phenomena at least in part mediated through changes in plasma volume status, and in what extent is currently unknown.

Limitations of our work are single center experience, retrospective study design and estimation of ePVS in one time point at the time of presentation. Also, no causal relationship can be inferred from observed associations. ePVS clinical correlations with clinical symptoms and other variables were evaluated at the time of hospital admission and not on the particular day from the onset of symptoms or on particular day of hospitalization. We were not able to appropriately address other timepoints as baseline due to analyzing registry level dataset that has the most complete information for the time of hospital admission. Since we included all consecutive patients with various comorbidities and various degrees of severity of COVID-19, they were heterogeneously treated with a number of possible therapies that may affect ePVS estimation. Effects of many medications, including corticosteroids and LMWH on ePVS dynamics and prognostic properties

in the context of COVID-19 is currently unknown. Due to investigated time period, vaccinated patients did not represent large proportion of hospitalized patients and we could not properly assess potential effects of vaccination and whether benefits observable among vaccinated patients with breakthrough infections might be mediated through changes in ePVS [27]. Strengths of our work are large cohort of mostly severe and critical, real-life elderly patients, representative of a tertiary-center experience. Our results provide valuable insight into associations of ePVS with clinical characteristics and imply potential importance of blood plasma volume evaluation in hospitalized COVID-19 patients. They raise questions whether specific therapies aimed at changing plasma volume and composition may improve prognosis of elderly patients with comorbidities presenting with viral pneumonia and may measuring/estimating blood plasma volume guide clinical decision making in this context. Further studies are needed.

In conclusion, higher ePVS, indicative of plasma volume expansion and inflammatory cytokine accumulation, may predispose respiratory deterioration, venous thromboembolism and death despite less severe initial clinical presentation. Lower ePVS, indicative of hemoconcentration, may predispose arterial thrombotic events. Both may be associated with higher mortality in hospitalized COVID-19 patients.

References:

[1] Wu Z, McGoogan JM (2020) Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. *JAMA : the journal of the American Medical Association,* **323,** 1239-42.

[2] Jurin I, Lucijanić M, Piskač Živković N, Lalić K, Zrilić Vrkljan A, Malnar Janeš L, et al. (2022) Incidence and risk factors for venous and arterial thromboses in hospitalized patients with coronavirus disease 2019: data on 4014 patients from a tertiary center registry. *Croatian medical journal,* **63,** 16-26.

[3] Connors JM, Levy JH (2020) Thromboinflammation and the hypercoagulability of COVID-19. *J Thromb Haemost,* **18,** 1559-61.

[4] Piskač Živković N, Lucijanić M, Bušić N, Jurin I, Atić A, Andrilović A, et al. (2022) The associations of age, sex, and comorbidities with survival of hospitalized patients with coronavirus disease 2019: data from 4014 patients from a tertiary-center registry. *Croat Med J,* **63,** 36-43.

[5] Otaki Y, Watanabe T, Konta T, Watanabe M, Asahi K, Yamagata K, et al. (2020) Impact of calculated plasma volume status on all-cause and cardiovascular mortality: 4-year nationwide community-based prospective cohort study. *PloS one,* **15,** e0237601.
[6] Lin Y, Xue Y, Liu J, Wang X, Wei L, Bai L, et al. (2021) Prognostic value of estimated plasma volume in patients with chronic systolic heart failure. *Journal of investigative medicine : the official publication of the American Federation for Clinical Research,* **69,** 338-44.
[7] Duarte K, Monnez JM, Albuisson E, Pitt B, Zannad F, Rossignol P (2015) Prognostic Value of Estimated Plasma Volume in Heart Failure. *JACC Heart failure,* **3,** 886-93.
[8] Chen J, Shen J, Cai D, Wei T, Qian R, Zeng C, et al. (2021) Estimated plasma volume status (ePVS) is a predictor for acute myocardial infarction in-hospital mortality: analysis based on MIMIC-III database. *BMC Cardiovasc Disord,* **21,** 530.
[9] Lucijanic M, Krecak I, Soric E, Sabljic A, Galusic D, Holik H, et al. (2022) MPN-541 Estimated Plasma Volume Status in Patients With Primary Myelofibrosis and Associated Thrombotic and Mortality Risks. *Clinical lymphoma, myeloma & leukemia,* **22 Suppl 2,** S341-s2.
[10] Krečak I, Zekanović I, Holik H, Morić Perić M, Coha B, Gverić-Krečak V (2022) Estimating plasma volume using the Strauss-derived formula may improve prognostication in polycythemia vera. *International journal of laboratory hematology,* **44,** e69-e71.
[11] Charlson ME, Pompei P, Ales KL, MacKenzie CR (1987) A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *Journal of chronic diseases,* **40,** 373-83.
[12] Subbe CP, Kruger M, Rutherford P, Gemmel L (2001) Validation of a modified Early Warning Score in medical admissions. *QJM : monthly journal of the Association of Physicians,* **94,** 521-6.
[13] World Health Organization (2020) Clinical management of COVID-19: interim guidance, 27 May 2020. Geneva: World Health Organization. Contract No.: WHO/2019-nCoV/clinical/2020.5.
[14] Lucijanic M, Piskac Zivkovic N, Ivic M, Sedinic M, Brkljacic B, Mutvar A, et al. (2021) Asymptomatic deep vein thromboses in prolonged hospitalized COVID-19 patients. *Wiener klinische Wochenschrift,* **133,** 1281-8.
[15] do Espírito Santo DA, Lemos ACB, Miranda CH (2020) In vivo demonstration of microvascular thrombosis in severe COVID-19. *Journal of Thrombosis and Thrombolysis,* **50,** 790-4.
[16] Liu H, Hu T, Zhang C, Chen X, Zhang S, Li M, et al. (2021) Mechanisms of COVID-19 thrombosis in an inflammatory environment and new anticoagulant targets. *Am J Transl Res,* **13,** 3925-41.
[17] Du F, Liu B, Zhang S (2021) COVID-19: the role of excessive cytokine release and potential ACE2 down-regulation in promoting hypercoagulable state associated with severe illness. *Journal of Thrombosis and Thrombolysis,* **51,** 313-29.
[18] Mondal R, Lahiri D, Deb S, Bandyopadhyay D, Shome G, Sarkar S, et al. (2020) COVID-19: Are we dealing with a multisystem vasculopathy in disguise of a viral infection? *Journal of Thrombosis and Thrombolysis,* **50,** 567-79.
[19] Lucijanic M, Bistrovic P, Jordan A, Mihaljevic I, Bukvic S, Kovacevic S, et al. (2023) Remdesivir use in severe and critical COVID-19 patients might be associated with lower incidence of arterial thrombotic events. *Am J Emerg Med,* **70,** 41-5.
[20] Rostami M, Mansouritorghabeh H (2023) Significance of heparin induced thrombocytopenia (HIT) in COVID-19: a systematic review and meta-analysis. *J Thromb Thrombolysis***,** 1-12.
[21] Pilia E, Belletti A, Fresilli S, Finco G, Landoni G (2022) Efficacy and safety of heparin full-dose anticoagulation in hospitalized non-critically ill COVID-19 patients: a meta-analysis of multicenter randomized controlled trials. *J Thromb Thrombolysis,* **54,** 420-30.
[22] Kobayashi M, Girerd N, Duarte K, Chouihed T, Chikamori T, Pitt B, et al. (2021) Estimated plasma volume status in heart failure: clinical implications and future directions. *Clin Res Cardiol,* **110,** 1159-72.
[23] Bakker J, Horowitz JM, Hagedorn J, Kozloff S, Kaufman D, Castro R (2021) Blood volume and albumin transudation in critically ill COVID-19 patients. *Crit Care,* **25,** 269.

[24] BROWN GE, KEITH NM (1924) BLOOD AND PLASMA VOLUME IN OBESITY. *Archives of Internal Medicine,* **33,** 217-23.
[25] Lemmens HJ, Bernstein DP, Brodsky JB (2006) Estimating blood volume in obese and morbidly obese patients. *Obes Surg,* **16,** 773-6.
[26] Vricella LK, Louis JM, Chien E, Mercer BM (2015) Blood volume determination in obese and normal-weight gravidas: the hydroxyethyl starch method. *Am J Obstet Gynecol,* **213,** 408.e1-6.
[27] Busic N, Lucijanic T, Barsic B, Luksic I, Busic I, Kurdija G, et al. (2022) Vaccination provides protection from respiratory deterioration and death among hospitalized COVID-19 patients: Differences between vector and mRNA vaccines. *J Med Virol,* **94,** 2849-54.

Table 1: The relationship between ePVS and individual comorbidities in hospitalized COVID-19 patients.

Comorbidity	**ePVS without comorbidity**	**ePVS with comorbidity**	**P value**
Arterial hypertension	4.69 (4.05-5.65)	4.8 (4.12-5.75)	**P=0.005 ***
Diabetes mellitus	4.69 (4.08-5.65)	4.88 (4.15-5.92)	**P<0.001 ***
Hyperlipoproteinemia	4.72 (4.08-5.7)	4.88 (4.17-5.87)	**P=0.003 ***
Obesity	4.84 (4.13-5.87)	4.65 (4.04-5.46)	**P<0.001 ***
Chronic kidney disease	4.69 (4.07-5.56)	5.56 (4.58-6.76)	**P<0.001 ***
Chronic heart failure	4.72 (4.085.65)	5 (4.21-6.09)	**P<0.001 ***
Coronary artery disease	4.75 (4.09-5.7)	4.92 (4.14-5.89)	**P=0.048 ***
Peripheral artery disease	4.73 (4.1-5.7)	5.2 (4.26-6.37)	**P<0.001 ***
GERD and ulcer disease	4.72 (4.08-5.65)	5.08 (4.27-6.2)	**P<0.001 ***
IBD	4.76 (4.1-5.73)	5.42 (4.51-6.57)	**P=0.003 ***
Chronic liver disease	4.76 (4.1-5.7)	5.48 (4.45-6.73)	**P<0.001 ***
Liver cirrhosis	4.76 (4.1-5.7)	6.21 (5.2-8.01)	**P<0.001 ***
Atrial fibrillation	4.76 (4.11-5.7)	4.81 (4.04-5.98)	P=0.424
VTE in anamnesis	4.76 (4.09-5.7)	5.16 (4.27-6.41)	**P<0.001 ***
CVI in anamnesis	4.76 (4.09-5.7)	4.95 (4.2-5.91)	**P=0.009 ***
MI in anamnesis	4.76 (4.1-5.73)	4.92 (4.16-5.8)	P=0.167
Epilepsy	4.76 (4.09-5.71)	5.28 (4.53-6.42)	**P<0.001 ***
Mental retardation	4.76 (4.1-5.73)	4.88 (4.24-6.04)	P=0.343
Dementia	4.72 (4.09-5.65)	4.95 (4.16-5.98)	**P<0.001 ***
Schizophrenia	4.76 (4.1-5.73)	5.04 (4.47-5.6)	P=0.116
Active malignancy	4.69 (4.05-5.52)	6.11 (5.04-7.47)	**P<0.001 ***

Comorbidity	**ePVS without comorbidity**	**ePVS with comorbidity**	**P value**
Thyroid disease	4.72 (4.08-5.69)	5.12 (4.32-6.17)	**P<0.001 ***
Autoimmune / rheumatic disease	4.76 (4.09-5.73)	5.08 (4.37-5.88)	**P=0.001 ***
COPD	4.76 (4.11-5.73)	4.75 (4.06-5.71)	P=0.564
Asthma	4.76 (4.11-5.73)	4.81 (4.02-5.76)	P=0.838
Chronic hemodialysis	4.73 (4.09-5.7)	6.27 (5.33-7.87)	**P<0.001 ***

*statistically significant at level P<0.05 / ePVS is shown as median and interquartile range. / Abbreviations: ePVS=estimated plasma volume status; GERD=gastroesophageal reflux disease; IBD=inflammatory bowel disease; VTE=venous thromboembolism; MI=myocardial infarction; CVI=cerebrovascular infarction; COPD=chronic obstructive pulmonary disease.

Table 2: Multivariate analyses of the association between ePVS stratified at quartiles and unfavorable clinical outcomes during hospitalization (logistic regression).

	Death	**MV**	**ICU**	**Arterial thrombosis**	**VTE**	**Major bleeding**	**Bacteriemia**
ePVS 2nd vs 1st quartile	P=0.052 OR=0.84 (0.7-1)	P=0.308 OR=1.11 (0.91-1.36)	P=0.714 OR=1.03 (0.86-1.24)	P=0.106 OR=0.77 (0.56-1.06)	P=0.936 OR=1.01 (0.74-1.39)	P=0.181 OR=1.41 (0.85-2.35)	P=0.574 OR=1.07 (0.84-1.37)
ePVS 3rd vs 1st quartile	**P=0.018 *** OR=0.81 (0.68-0.96)	**P=0.027 *** OR=1.25 (1.03-1.53)	**P=0.025 *** OR=1.23 (1.03-1.47)	P=0.054 OR=0.74 (0.54-1.01)	P=0.475 OR=1.12 (0.82-1.53)	P=0.156 OR=1.45 (0.87-2.4)	P=0.154 OR=1.19 (0.94-1.52)
ePVS 4th vs 1st quartile	**P=0.038 *** OR=1.21 (1.01-1.45)	**P=0.002 *** OR=1.39 (1.13-1.72)	**P<0.001 *** OR=1.44 (1.19-1.74)	**P=0.002 *** OR=0.6 (0.43-0.82)	**P=0.003 *** OR=1.62 (1.18-2.2)	**P<0.001 *** OR=3.86 (2.46-6.07)	**P=0.018 *** OR=1.35 (1.05-1.74)
Age (years)	**P<0.001 *** OR=1.05 (1.04-1.05)	**P=0.025 *** OR=0.99 (0.99-1)	**P<0.001 *** OR=0.99 (0.98-0.99)	P=0.835 OR=1.0 (0.99-1.01)	P=0.891 OR=1.0 (0.99-1.01)	P=0.923 OR=1.0 (0.99-1.01)	**P=0.005 *** OR=0.99 (0.98-1)

	Death	**MV**	**ICU**	**Arterial thrombosis**	**VTE**	**Major bleeding**	**Bacteriemia**
Male sex	**P<0.001 *** OR=1.32 (1.16-1.51)	**P<0.001 *** OR=1.71 (1.46-1.99)	**P<0.001 *** OR=1.66 (1.44-1.9)	P=0.909 OR=0.99 (0.78-1.25)	P=0.076 OR=0.82 (0.65-1.02)	**P=0.043 *** OR=1.38 (1.01-1.88)	**P<0.001 *** OR=1.59 (1.32-1.91)
Severe or critical COVID-19	**P<0.001 *** OR=4.43 (3.88-5.06)	**P<0.001 *** OR=3.9 (3.4-4.46)	**P<0.001 *** OR=3.17 (2.81-3.57)	**P=0.003 *** OR=0.8 (0.7-0.93)	**P<0.001 *** OR=1.56 (1.32-1.84)	**P=0.022 *** OR=1.28 (1.04-1.59)	**P<0.001 *** OR=2.17 (1.88-2.51)
Charlson comorbidity index	**P<0.001 *** OR=1.22 (1.18-1.26)	P=0.191 OR=1.02 (0.99-1.06)	P=0.084 OR=1.03 (1-1.06)	**P<0.001 *** OR=1.18 (1.12-1.23)	**P=0.004 *** OR=0.92 (0.87-0.97)	P=0.271 OR=1.04 (0.97-1.1)	P=0.269 OR=1.02 (0.98-1.07)

*statistically significant at level P<0.05 / Abbreviations: ePVS=estimated plasma volume status; MV=mechanical ventilation; ICU=intensive care unit; VTE=venous thromboembolism.

Figure 1: Baseline associations between ePVS quartiles and **A)** laboratory parameters of inflammation (C reactive protein – CRP, interleukin-6 – IL-6, D-dimers and ferritin), and **B)** parameters representative of cellular components of the blood (white blood cells – WBC, hemoglobin and platelet count) (* = statistically significant at level $P<0.05$).

Figure 2: Rates of unwanted outcomes during hospitalization stratified according to the ePVS quartiles (* = statistically significant at level $P<0.05$).