Letter to the Editor

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Homocysteine (Hcy) assessment to predict outcomes of hospitalized Covid-19 patients: a multicenter study on 313 Covid-19 patients

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To the Editor,

The Covid-19 pandemic is a global challenge, with rapidly increasing cases from the high infective aetiological agent, the Covid-19 virus. In October 2020, over 48 million confirmed Covid-19 cases and one million deaths were confirmed (www.who.int). There is an urgent need to identify predictive clinical, epidemiological, genetic and laboratory markers for outcomes, especially regarding microvasculature damage, potentially safely treated with therapeutic interventions. Homocysteine (Hcy) has recently been proposed as a potential predictive biomarker for Covid-19 infection severity [1, 2].

SARS-CoV-2 transfers methyl group for viral RNA capping from the host cell S-adenosylmethionine (SAM), converted into S-adenosylhomocysteine (SAH). SAH hydrolase (SAHH) removes adenosine from SAH, and produces an intermediate product “homocysteine”, recycled by the remethylation and trans-sulphuration pathway in the human body [3]. In cardiovascular patients, Hcy levels are used as predictive markers of thromboembolic risk, but has not yet been applied to Covid-19 risk stratification. This study aims to evaluate the predictive role of plasma Hcy as a prognostic marker of Covid-19 patients’ outcome. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and Good Clinical Practices and, in compliance with local regulatory requirements.

A multicenter, retrospective analysis, including patients hospitalized for Covid-19 between April 2020 and September 2020, was performed. Hcy levels were determined using chemiluminescent microparticle immunoassay (Architect Homocysteine assay, Abbott). Venous blood samples were collected upon hospitalization according to standard hospital procedures. Routine laboratory parameters obtained are outlined in Table 1. Patient clinical information and one month survival status after Covid-19 diagnosis were recorded.

Table 1: Descriptive demographic and clinical features of hospitalized Covid-19 patients. A comparison between survivors and non-survivors characteristics and univariate analysis of predictive markers for in-hospital mortality.

Demographic and clinical features:	Total (n=313)	Status[a]		Univariate analysis		
		Non-survivors (n=34. 11.2%)	Survivors (n=270. 86.3%)	OR	95% CI	p-Value
Gender, M	204(65.2)	22(64.7)	175(64.8)	0.89	(0.50–1.57)	0.69
Age, median (1Q–3Q)	62(50–74)	73(64–78)	60(49–73)	1.00	(1.01–1.04)	0.002
Citizenship[b]						
Italians	256(82.8)	34	213	ref		
Foreigners	53(17.2)	0	25	1.57	(0.70–3.53)	0.271
Status[a]						
Survivor	270(86.2)	–	270(100)	ref		
Non-survivor	34(10.9)	34(100)	–	3.08	(1.45–6.51)	0.003
Unknown	9(2.9)	–	–			
Ln homocysteine, μmol/L[c]	2.4 ± 0.5(−0.4–4.4)	2.6 ± 0.8(1.4–4.4)	2.4 ± 0.5(−0.4–3.9)	–		
Ln D-dimer, μg/L[c]	6.7 ± 1.4(2.3–10.6)	7.7 ± 0.9(5.9–9.5)	6.5 ± 1.3(2.3–10.5)	1.05	(0.61–1.29)	0.605
Ln PT, s	3.4 ± 0.8(−0.6–4.7)	3.8 ± 0.4(3.2–4.7)	3.4 ± 0.8(−0.6–4.7)	0.79	(0.55–1.14)	0.207
Ln aPTT, s	1.4 ± 1.7(−3.1–4.6)	1.3 ± 1.8(−0.1–4.6)	1.3 ± 1.7(−3.1–3.8)	0.86	(0.72–1.04)	0.121
Ln fibrinogen, g/L	1.4 ± 0.4(−1.6–2.7)	1.5 ± 0.4(0.8–2.3)	1.4 ± 0.4(−1.6–2.7)	0.74	(0.41–1.35)	0.326
Ln BNP, pmol/L[c]	−2.2 ± 1.7(−5.3–3.7)	−0.7 ±1–0.7(−3.1–3.3)	−2.3 ± 1.7(−5.3–3.7)	1.33	(1.11–1.59)	0.002
Ln CK, nmol/(s·L	7.1 ± 1.1(3.5–11.2)	7.3 ± 1.4(5.6–10.5)	7.1 ± 1.1(3.5–11.2)	0.89	(0.69–1.14)	0.342
Ln troponin, ng/L[c]	−0.5 ± 3.6(−6.9–7.7)	2.3 ± 3.5(−5.8–7.7)	−1.0 ± 3.5(−6.9–7.6)	0.99	(0.91–1.07)	0.785
Ln red blood cells, $\times 10^6$/L	1.4 ± 0.5(−1.8–6.2)	1.3 ± 0.2(0.6–1.9)	1.4 ± 0.6(−1.8–6.2)	1.00	(0.63–1.61)	0.979
Ln white blood cells, $\times 10^9$/L	1.9 ± 0.6(−1.7–4.7)	2.1 ± 0.6(0.4–3.2)	1.9 ± 0.6(−1.7–4.7)	1.10	(0.72–1.67)	0.672
Ln neutrophils, $\times 10^9$/L[c]	1.7 ± 0.7(−0.3–4.7)	1.9 ± 0.7(0.3–3.0)	1.7 ± 0.7(−0.4–4.7)	1.22	(0.82–1.81)	0.32
Ln lymphocytes, $\times 10^9$/L[c]	0.1 ± 0.7(−2.2–4.9)	−0.3 ± 0.6(−2.2–−0.5)	0.1 ± 0.7(−1.7–4.9)	0.99	(0.68–1.44)	0.953
Ln neutrophils/lymphocytes, $\times 10^9$/L[c]	1.5 ± 0.9(−2.9–4.6)	2.3 ± 0.8(0.3–3.8)	1.4 ± 0.9(−2.9–4.6)	1.12	(0.84–1.51)	0.414
Ln monocytes, $\times 10^9$/L[c]	−0.8 ± 0.7(−3.5–2.2)	−1.1 ± 1.0(−3.5–0.1)	−0.8 ± 0.7(−2.9–2.2)	1.30	(0.91–1.87)	0.153
Ln monocytes/lymphocytes, $\times 10^9$/L	−0.9 ± 0.7(−5.2–1.7)	−0.7 ± 0.8(−2.3–0.4)	−1.0 ± 0.7(−5.2–1.7)	1.33	(0.91–1.93)	0.137
Ln eosinophils, $\times 10^9$/L	−2.9 ± 1.1(−5.5–0.1)	−3.3 ± 1.3(−4.6–0.1)	−2.9 ± 1.1(−5.5–0.2)	1.09	(0.81–1.47)	0.562
Ln basophil, $\times 10^9$/L	−3.6 ± 1.3(−6.9–6.1)	−3.3 ± 2.0(−4.6–5.7)	−3.6 ± 1.2(−6.9–6.1)	0.94	(0.70–1.25)	0.656
Ln platelet, $\times 10^9$/L	5.4 ± 0.6(−2.3–6.6)	5.2 ± 0.6(3.1–6.1)	5.4 ± 0.6(−2.3–6.6)	0.80	(0.55–1.18)	0.26

PT, prothrombin time; PTT, partial thromboplastin time; BNP, brain natriuretic peptide; CK, creatine kinase. [a]Nine cases unknown. [b]Four data missing. [c]p-Value<0.05, statically significant. Data are presented as mean ± SD and number (n) of patients (%), as appropriate.

Statistical analysis was performed using STATA®, version 14. Descriptive statistics for baseline demographic and clinical characteristics was performed and normality of data distribution was assessed with the Kolmogorov–Smirnov test. Natural log transformation (ln) was calculated if data were not normally distributed; log-transformed means and standard deviations (SD) were determined. Continuous variables were compared between subgroups using Unpaired Student's t test and categorical variables using Pearson's chi-squared test. Association between parameters and outcome was assessed with univariate and multivariate logistic regression models with stepwise forward selection. Hcy cut off value for in-hospital mortality prediction was determined by receiver operating characteristic (ROC) curve; maximum Youden's index value "sensitivity + specificity – 1". p<0.05 was considered statistically significant.

The study included 313 patients, mostly male (65.2%) of 62 years average age. Most patients (86.2%) survived; nine patients (2.9%) were transferred to other hospitals and lost to follow up (Table 1). According to outcome, age, Italian nationality, D-Dimer, PT, troponin, lymphocyte count and neutrophil/lymphocytes count ratio were significantly associated with non-survival of Covid-19 infection. Plasma Hcy levels were significantly higher in non-survivors (Table 1).

Logistic regression analysis revealed that increasing age (OR 1.04), Hcy levels (OR 1.06), and Neutrophil/Lymphocyte count ratio (BNP) (OR 1.03) were associated with hospital mortality risk (p<0.05). Multivariate analysis of the study population adjusted for age and gender, demonstrated that Hcy and Troponin are predictors of severe-progression (p<0.05).

The optimal cut-off for Hcy as predictive of in-hospital mortality was estimated to be >16 μmol/L; sensitivity and specificity were 41 and 83%, respectively (see Figure 1); the area under the curve is 0.55. Patients grouped according to Hcy levels below and above the 16 μmol/L cut-off revealed a significantly association with in-hospital mortality (p=0.002), increasing age (p=0.004) and BNP (p<0.004) (data not shown).

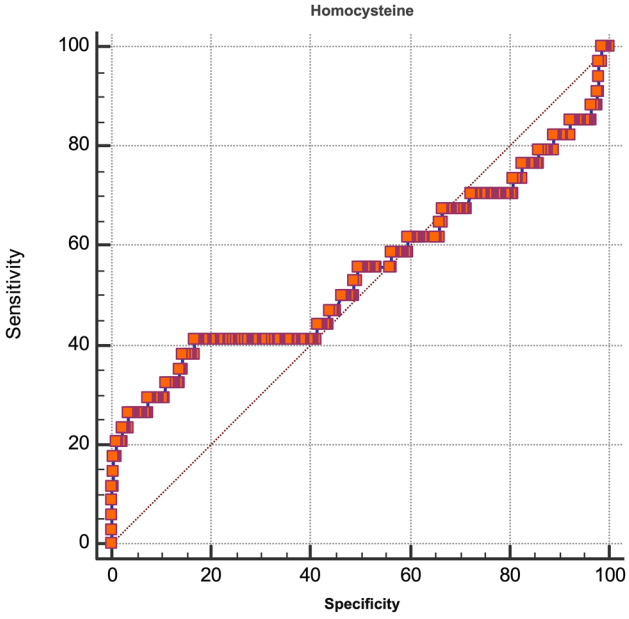

Figure 1: Receiver operating characteristic (ROC) curve for predicting in-hospital mortality using the homocysteine concentration. Area under the curve is 0.55 (cut-off: >16.4, Se: 41%, Sp: 83%).

Our results demonstrate that Hcy is a predictive marker for hospitalized Covid-19 patients' outcome. Plasma Hcy levels correlate significatively both as a continuous value and dichotomic value, with an optimal cut-off of 16 µmol/L.

In a previous study, including 273 Covid-19 patients, hyper-homocysteinemia was reported to be predictive for computed tomography (CT)-imaging lung progression. Among 40 parameters, age, Hcy and monocyte/lymphocyte ratio (MLR) were found to be significant predictors of disease progression; patients with hyper-homocysteinemia (>15.4 µmol/L) had a three-fold increased risk. Of these three predictive markers, Hcy is the only readily modifiable marker.

Several studies have elucidated the pathogenic correlation between Covid-19 infection and Hcy metabolism. Recently, it was reported that regulatory pathways directly involved with Hcy activates angiotensin II type I receptor [4]. Ferroptosis, a recently discovered form of controlled cell death, is characterized by lipid and iron reactive oxygen species accumulation and smaller mitochondria with condensed mitochondrial membrane densities. It is linked to neurological disorders, including cognitive impairment, ageusia and anosmia (taste and smell loss); common manifestations of Covid-19 disease [5].

Several recent cohort studies have investigated the presence of reliable prognostic biomarkers for the progression, severity and mortality of Covid-19 hospitalized patients [6]. According to our results, other significant ($p<0.05$) biomarkers were RBC (OR 0.68) and Lymphocytes count (OR 0.23) that were protective and ultimately associated with COVID-19 survival (Table 1).

The observation that *MTHFR* genetic polymorphisms among different populations correlate with an increased incidence and severity of Covid-19 infection will facilitate predicting population-based risk factors and assist in the implementation of diagnostic and therapeutic interventions for patients, who may benefit from Vitamin B and Folic acid supplements [2].

It may be possible to provide specific vitamin supplementation programs, reducing Hcy levels in populations with poor dietary regimens and/or high prevalence of *MTHFR* 677T allele.

The supplementation of vitamin B9 and other vitamins of the same group (for example B12) has been demonstrated to normalize blood Hcy levels, both in apparently healthy individuals and patients with a history of stroke or Parkinson's disease [7]. It is reasonable to suggest that proper integration of vitamin B and Folic acid, could have protective clinical effects for patients with infectious disease, associated with *MTHFR* 677T allele or other pathologic conditions.

Hyper-homocysteinemia is attributed to many virus infections, including human hepatitis, papilloma [8] and immunodeficiency [9] viruses. B- vitamins (B2, B3 and B6) are associated with the enhancement of the immune system [10].

The current study is limited by the inclusion of patients with multiple comorbidities from chronic illnesses (data not shown), hospitalized with Covid-19 infection. The results cannot therefore be generalized to all Covid-19 patients.

If confirmed by larger studies, plasma Hcy levels and *MTHFR* gene sequencing may become routine markers for clinical management of Covid-19 infection, as well as an important clinical target that should be normalized through vitamin and nutrient supplementation (Folic acid and Vitamin B12).

It is therefore reasonable to conclude that the clinical management of Covid-19 infection may be improved by early determination of a signature of several biomarkers (including biochemical, hematological, genetic and metabolic), to monitor therapeutic intervention efficacy and/or predict the clinical course of the Covid-19 disease. For both infected patients and/or those awaiting diagnostic confirmation of Covid-19 disease, these biomarkers may predict risk stratification and determine patient management.

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