Predicting COVID-19 Hospitalized Patients’ Outcome with Homocysteine

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Received date: March 08, 2021, Accepted date: May 21, 2021

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Keywords: Homocysteine (Hcy), COVID-19 vulnerability, Predictor parameters, MTHFR gene, Biomarkers, MTHFR677C>T mutations

In October 2020, we published ‘Homocysteine as a potential predictor of cardiovascular risk in patients with COVID-19’ [1]. Since then, recent scientific evidence from other authors [2-6], together with our own continuing research to include a larger cohort of hospitalized COVID-19 patients [7], has supported our original hypothesis, confirming that homocysteine (Hcy) is a predictive marker of COVID-19 outcome.

The COVID-19 pandemic has provoked a global, rapid increase of cases due to the high infectivity of the etiological agent, COVID-19 virus. In February 2021, over 110 million confirmed COVID-19 cases with 1 million deaths were reported worldwide (www.who.int).

Since the beginning of the pandemic, the identification of reliable biomarkers for COVID-19 disease progression has been a great challenge. Among various biomarkers tested, Hcy has sparked particular interest due to its association with both the metabolism of the SARS-CoV-2 virus and cardiovascular complications, which have proven to be the main cause of death among COVID-19 patients [8-10]. It is known that the SARS-CoV-2 virus 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 called “homocysteine,” which is recycled by remethylation and the trans-sulphuration pathway in the human body [5,6,11,12].

Recently, novel regulatory mechanisms directly involved with Hcy in the activation of angiotensin II type receptor have been described [13]. Ferroptosis, a newly identified form of regulated cell death, does not share morphological, biochemical, or genetic similarities with other forms of regulated cell death, such as apoptosis [14]. It is characterized by the accumulation of iron and lipid reactive oxygen species (ROS) and by smaller mitochondria with condensed membrane densities. Increasing evidence suggests that ferroptosis dysfunction is positively related to several human diseases, including tumorigenesis [15]. Ferroptosis was found to be linked to common symptoms of COVID-19 disease, namely neurological disturbances, including cognitive impairment, ageusia, and anosmia.
Hcy has been reported as a potential predictive biomarker for COVID-19 infection severity in many studies [1-4]. In a series of 273 Chinese hospitalized patients with mild COVID-19 disease, over 40 parameters were measured at admission. Disease progression was registered for 72 patients (computed tomography [CT] lung scans) and age, Hcy plasma levels and monocyte-to-lymphocyte ratio (MLR) were the only significant predictors in hyperhomocysteinemic patients (>15.4 µmol/L), estimated to correspond with a three-fold increased risk of disease evolution at radiological images. Interestingly, Hcy is the only predictive marker identified which is readily modifiable. Further, recent data confirmed the value of Hcy (together with age, MLR, and time from disease onset to hospital admission) in predicting the risk of severe pneumonia (on chest CT scan). The authors did not report any other additional organ involvement [3].

Our retrospective cohort study, including 313 COVID-19 hospitalized patients (female 34.8%; mean age 62 years) between April-September 2020, also included a broad panel of clinical laboratory data collected at admission. Of the enrolled patients, 10.9% died during hospitalization (3% were transferred to other hospitals and were lost to follow-up). Hcy was found to be the strongest predictor of Covid-19 critical-progression leading to death. Univariate analysis demonstrated that age (OR 1.04), Hcy (OR 1.06), and Neutrophil/Lympocyte count ratio (OR 1.03) were significant predictors of critical progression leading to death and RBC (OR 0.68) and Lymphocytes count (OR 0.23) with benign outcome (Table 1). ROC analysis

| Epidemiologic and clinical characteristics: | Status* | Univariate Analysis |
|--------------------------------------------|---------|--------------------|
| Gender, M                                  | Total (n=313) | Dead (n=34. 11.2%) | Alive (n=270. 86.3%) |
|                                            | OR       | 95% CI             | p-value               |
| Gender, M                                  | 204 (65.2) | 22 (64.7)          | 175 (64.8)            |
| Gender, M                                  | 0.89     | (0.50-1.57)        | 0.690                 |
| Age, Median (1Q-3Q)                         | 62 (50-74) | 73 (64-78)         | 60 (49-73)            |
| Age, Median (1Q-3Q)                         | 1.02     | (1.01-1.04)        | 0.002                 |
| Citizenship                                | 256 (82.8) | 34              | 213     |
| Citizenship                                | ref      |                   |                      |
| Italians                                   | 53 (17.2) | 0               | 25        |
| Italians                                   | 1.57     | (0.70-3.53)        | 0.271                 |
| Foreigners                                 | 9 (2.9)  | -               | -         |
| Foreigners                                 | 3.08     | (1.45-6.51)        | 0.003                 |
| Status*                                    |          |                   |                      |
| Alive                                      | 270 (86.2) | -               | 270 (100)         |
| Alive                                      | ref      |                   |                      |
| Dead                                       | 34 (10.9) | 34 (100)         | -         |
| Dead                                       | 3.08     | (1.45-6.51)        | 0.003                 |
| Unknown                                    | 9 (2.9)  | -               | -         |
| Ln Homocysteine (µmol/L)                   | 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 Homocysteine (µmol/L)                   | 1.05     | (0.61-1.29)        | 0.605                 |
| Ln D-dimer (ng/mL)                         | 6.7 ± 1.4 (2.3-10.6) | 7.7 ±9.0 (5.9-9.5) | 6.5 ±1.3 (2.3-10.5) |
| Ln D-dimer (ng/mL)                         | 1.05     | (0.61-1.29)        | 0.605                 |
| Ln PT (s)                                  | 1.4 ± 1.7 (-3.1-4.6) | 3.8 ±0.4 (3.2-4.7) | 3.4 ±0.8 (-0.6-4.7) |
| Ln PT (s)                                  | 0.79     | (0.55-1.14)        | 0.333                 |
| 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) |
| Ln aPTT (s)                                | 0.86     | (0.72-1.04)        | 0.121                 |
| Ln Fibrinogen (mg/dL)                      | 6.0 ± 0.4 (2.9-7.3) | 6.1 ± 0.4 (4.6-6.9) | 6.0 ± 0.4 (2.9-7.3) |
| Ln Fibrinogen (mg/dL)                      | 0.74     | (0.41-1.35)        | 0.326                 |
| Ln BNP (pmol/mL)                           | 4.6 ± 1.7 (1.65-10.6) | 6.2 ± 1.7 (3.7-10.2) | 4.5 ± 1.7 (1.6-10.6) |
| Ln BNP (pmol/mL)                           | 1.33     | (1.11-1.59)        | 0.002                 |
| Ln CK (U/L)                                | 4.3 ± 1.1 (0.6-8.4) | 4.5 ± 1.4 (2.8-7.7) | 4.3 ± 1.1 (0.6-8.4) |
| Ln CK (U/L)                                | 0.89     | (0.69-1.14)        | 0.342                 |
| Ln Troponin (ng/L)                         | -0.5 ± 3.6 (-6.9-7.7) | 2.3 ± 0.8 (0.4-3.2) | -1.0 ± 3.5 (-1.8-6.2) |
| Ln Troponin (ng/L)                         | 0.99     | (0.91-1.07)        | 0.785                 |
| Ln Red Blood Cells (10⁶/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) |
| Ln Red Blood Cells (10⁶/L)                 | 1.00     | (0.63-1.61)        | 0.979                 |
| Ln White Blood cells (10⁹/L)               | 1.9 ± 0.6 (-1.7-4.7) | 2.1 ± 0.6 (0.0-3.2) | 1.9 ± 0.6 (-1.7-4.7) |
| Ln White Blood cells (10⁹/L)               | 1.10     | (0.72-1.67)        | 0.672                 |
| Ln Neutrophils (10⁹/L)                     | 1.7 ± 0.7 (-0.3-4.7) | 1.9 ± 0.7 (0.3-3.0) | 1.4 ± 0.9 (-2.9-4.6) |
| Ln Neutrophils (10⁹/L)                     | 1.22     | (0.82-1.81)        | 0.320                 |
indicated Hcy cut off of 16 µmol/L for predicting COVID-19 infection outcome (sensitivity 40% and specificity 84%); patients with Hcy levels >16 µmol/L had significantly increased risk of in-hospital mortality (p=0.002) both as a continuous and dichotomic value. Our results demonstrate that Hcy is an effective predictive biomarker for hospitalized COVID-19 patients’ outcome.

Several studies have demonstrated the importance of vitamin supplementation in patients with the COVID-19 disease. Vitamin B supplements (especially B9 and B12) are able to normalize blood Hcy levels in both apparently healthy individuals and patients with a history of stroke or Parkinson’s disease [18-20]. It is reasonable to suggest that B vitamins and Folic acid integration may have protective clinical effects for patients with infectious disease, due to MTHFR genetic polymorphisms of MTHFR C677T and COVID-19 incidence and mortality rates seems to be intriguing; it may be useful biomarker COVID-19 infection severity stratification and it may be used for preventive medical treatments and supplementations.

Hyper-homocysteinemia is related to many virus infection outcomes, including human hepatitis virus [21], human papilloma virus [22] and Human immunodeficiency virus [23,24]. B vitamins (B2, B3 and B6) have a key role in the enhancement of the immune system [25].

Even though Hcy has been proven as a critical biomarker of cardiovascular risk and complications in hospitalized COVID-19 patients, it has not yet been adopted in prospective studies for useful laboratory markers for the stratification of COVID-19 patients.

Hcy may be a valuable biomarker which can help clinicians identify patients who are at higher risk for severe COVID-19 infection. Hcy plasma levels are easily determined by a simple and affordable laboratory test.

The association between Hcy levels >16 µmol/L and worse COVID-19 prognosis should encourage preventive health programs aimed to supplement dietary group B vitamins and folic acid for COVID-19 patients.

Table 1: Descriptive epidemiological 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.

| Table 1: Descriptive epidemiological 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. |
|-----------------------------------------------|
| Ln Lymphocytes (10^9/L) | 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) | 0.153 |
| Ln Neutrophils/ Lymphocytes | 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) | 0.141 |
| Ln Monocytes (10^9/L) | -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 | -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 (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) | 1.07 | (0.81-1.47) | 0.562 |
| Ln Basophils (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 (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 | (0.95-1.18) | 0.260 |

*9 cases unknown; b4 data missing; Data are presented as mean ± SD and number (n) of patients (%), as appropriate.

References

1. Ponti G, Ruini C, Tomasi A. Homocysteine as a potential predictor of cardiovascular risk in patients with COVID-19. Medical Hypotheses. 2020 Oct;143:109859.
2. Ponti G, Maccaferri M, Ruini C, Tomasi A, Ozben T. Biomarkers associated with COVID-19 disease progression. Crit Rev Clin Lab Sci. 2020:1-11.
3. Yang Z, Shi J, He Z, Lü Y, Xu Q, Ye C, et al. Predictors for imaging progression on chest CT from coronavirus disease 2019 (COVID-19) patients. Aging (Albany NY). 2020 Apr 15;12 (7):6037.
4. Ibrahimagić OĆ, Smajlović D, Dostović Z, Vidović M, Tupković E, Kunić S. Comment On An Article: “Homocysteine as a potential predictor of cardiovascular risk in patients with COVID-19”. Med Hypotheses. 2020;143:110107.
5. Singh Y, Gupta G, Satija S, Negi P, Chellappan DK, Dua K. RAAS blockers in hypertension posing a higher risk towards the COVID-19. Dermatologic Therapy. 2020: e13501.
6. Mahalapbutr P, Kongtaworn N, Rungrotmongkol T. Structural insight into the recognition of S-adenosyl-L-homocysteine and sinefungin in SARS-COV-2 NSP16/NSP10 RNA cap 2′-O-methyltransferase. Computational and Structural Biotechnology Journal. 2020 Jan 1;18:2757-65.
7. Ponti G, Roli L, Oliva G, Manfredini M, Trenti T, Kaleci S, et al. Homocysteine (Hcy) assessment to predict outcomes of hospitalized COVID-19 patients: a multicenter study on 313 COVID-19 patients. Clinical Chemistry and Laboratory Medicine (CCLM). 2021 Mar 25.
8. Annamaria V, Rosetta R, Chiara C, Giuseppina B. COVID-19 and cardiovascular consequences: Is the endothelial dysfunction the hardest challenge? Thrombosis Research. 2020;196:143-51.

9. Dhakal BP, Sweitzer NK, Indik JH, Acharya D, William P. SARS-CoV-2 infection and cardiovascular disease: COVID-19 heart. Heart, Lung and Circulation. 2020;29:973-87.

10. Nishiga M, Wang DW, Han Y, Lewis DB, Wu JC. COVID-19 and cardiovascular disease: from basic mechanisms to clinical perspectives. Nature Reviews Cardiology. 2020 Sep;17(9):543-58.

11. Rosas-Lemus M, Minasov G, Shuvalova L, Inniss NL, Kiryukhina O, Brunzelle J, et al. High-resolution structures of the SARS-CoV-2 2′-O-methyltransferase reveal strategies for structure-based inhibitor design. Science Signaling. 2020 Sep 29;13(651).

12. Gurung AB. In silico structure modelling of SARS-CoV-2 Nsp13 helicase and Nsp14 and repurposing of FDA approved antiviral drugs as dual inhibitors. Gene Reports. 2020 Dec 1;21:100860.

13. Osaki T, Ohshima M, Tomita Y, Matsugi N, Nomura Y. Clinical and physiological investigations in patients with taste abnormality. Journal of Oral Pathology & Medicine. 1996 Jan;25(1):38-43.

14. Fumagalli C, Rozzini R, Vannini M, Coccia F, Cesaroni G, Mazzeo F, et al. Clinical risk score to predict in-hospital mortality in COVID-19 patients: a retrospective cohort study. BMJ Open. 2020 Sep 1;10(9):e040729.

15. Lu B, Chen XB, Ying MD, He QJ, Cao J, Yang B. The role of ferroptosis in cancer development and treatment response. Frontiers in Pharmacology. 2018 Jan 12;8:992.

16. Edeas M, Saleh J, Peyssonnaux C. Iron: Innocent bystander or vicious culprit in COVID-19 pathogenesis?. International Journal of Infectious Diseases. 2020 Aug 1;97:303-5.

17. Henry BM, De Oliveira MH, Benoit S, Plebani M, Lippi G. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. Clinical Chemistry and Laboratory Medicine (CCLM). 2020 Jun 25;58(7):1021-8.

18. Malinow MR, Bostom AG, Krauss RM. Homocyst(e)ine, diet, and cardiovascular diseases: a statement for healthcare professionals from the Nutrition Committee, American Heart Association. Circulation. 1999 Jan 12;99(1):178-82.

19. Belcastro V, Pierguidi L, Castrioto A, Menichetti C, Gorgone G, Ientile R, et al. Hyperhomocysteinemia recurrence in levodopa-treated Parkinson’s disease patients. European Journal of Neurology. 2010 May;17(5):661-5.

20. Ibrahimagic OC, Smajlovic D, Dostovic Z, Pasic Z, Kunic S, Iljazovic A, et al. Hyperhomocysteinemia and its treatment in patients with parkinson’s disease. Materia Socio-medica. 2016 Jul 24;28(4):303.

21. Adinolfi LE, Ingrassio D, Cesar G, Cimmino A, D’Antò M, Capasso R, et al. Hyperhomocysteinemia and the MTHFR C677T polymorphism promote steatosis and fibrosis in chronic hepatitis C patients. Hepatology. 2005 May;41(5):995-1003.

22. Abike F, Engin AB, Dunder I, Tapisiz OL, Aslan C, Kuthlay L. Human papilloma virus persistence and neopterin, folate and homocysteine levels in cervical dysplasias. Archives of Gynecology and Obstetrics. 2011 Jul;284(1):209-14.

23. Demince R, Silva TC, de Oliveira VH. Elevated homocysteine levels in human immunodeficiency virus-infected patients under antiretroviral therapy: A meta-analysis. World Journal of Virology. 2015 May 12;4(2):147.

24. Roblin X, Pofelski J, Zarski JP. Steatosis, chronic hepatitis virus C infection and homocysteine. Gastroenterologie Clinique et Biologique. 2007 Apr 1;31(4):415-20.

25. Zhang L, Liu Y. Potential interventions for novel coronavirus in China: A systematic review. Journal of Medical Virology. 2020 May;92(5):479-90.