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Elevated Methaemoglobin Level in COVID-19 Patients in Intensive Care Unit

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Abstract

There have been reports that methaemoglobin levels are raising in COVID-19 patients.

Methods: The diagnosis of Coronavirus-19 infection was confirmed by RT-PCR. The methaemoglobin determination was by cyanid derivative spectrophotometry.

Results: There were altogether 46 patients (11 male, 35 female) included. Their median age was 70 y. (29-89). The methaemoglobin median value was 3,9 15 of 46 patients died. The methaemoglobin median value of departed patients was 8%, and 2,5% was among survivors. (p=0,001).19 patients were blood transfused. Their methaemoglobin median was 11%, otherwise the non transfused patients presented 2,7% methaemoglobin median.(p=0,001).We performed two binary logistic regression calculation, computing the elevated methaemoglobin level as an independent risk factor for the mortality. The predictor of methaemoglobin was 0,062 the constant -1,266 the odds ratio 1,06. The other binary logistic regression tested the fact of transfusion for the mortality. Predictor of transfusion 1,1474, constant -1,2527 odds ratio 3,15.

Conclusion: The correlation of methaemoglobin with mortality is worth examining further. The role of transfusion is obscure, because the methaemoglobin can be elevated in blood conserves during storage. Testing the methemoglobin seems to be important, but the pathomechanism needs further research.

Keywords: COVID-19; Methaemoglobin level; Transfusion; Mortality

Introduction

The COVID-19 pandemic made several new diagnostic approaches necessary. Among them, methods assessing the short-term mortality risks were prominent. An example of this is the kinetics of elevated D dimer. Some papers raised the potential predictive value of elevated methaemoglobin level. Methaemoglobin is a haemoglobin derivative, where the 2-times positive iron ion is oxidized to the 3-times positive form in the porphyrine ring. This (ferri) modification is unable to transport oxygen. The significance and mechanism of methaemoglobin formation were published more than one hundred years ago [1]. Some medical case reports and a review formed a hypothesis about the possible correlation between an elevated methaemoglobin level and mortality in COVID-19 patients [2-4].

These papers tried to find causality between the methaemoglobin level and increased mortality. At that time, treatment with chloroquine was the most integral part of the protocol. The harmful effect of anti-malarial drugs on haemoglobin structure is a well-known fact. After stopping the application of anti-malarial drugs, an article was published reporting an elevated methaemoglobin level in COVID-19 patients. The authors blamed azythromycin for the phenomenon. Azythromycin is able to oxidize the haemoglobin molecule.

Unfortunately, the insufficiently standardized methodology makes it difficult to judge the significance of elevated methaemoglobin. Pulse-oximetry is cheap and common, but some papers proved its efficacy [4,5]. There are CO-oximeters on the market, but their optics and software are heterogeneous with respect to the numbers and types of wavelengths and elimination of lipid interference [6-9]. During our work, we applied the determination of methaemoglobin *via* its cyanide derivatives [10], regarding the fact that the Drabkin method is the gold standard in haemoglobin determination. Later, this method was improved, and some pitfalls were eliminated [11].

Materials and Methods

Patients method

In January and February, 2022, 46 patients' results could be evaluated. No patients were vaccinated. There were 11 male and 35 female patients. Their median age was 70 years (interquartile range: 49-80 yrs; youngest: 29 yrs; oldest: 89 yrs.). For two patients, airway pressure release ventilation was applied (Table 1). One patient received synchronized intermittent mandatory ventilation. Local anaesthesia was provided (lidocaine) (Table 1). All patients received amoxycilin/clavulanic acid and chlarithromycin antibiotic treatment. Azithromycin-treated patients are indicated in the table. Every patient received LMWH treatment and 5 mg dexamethasone. SARS-2 positivity was confirmed from nasopharyngeal sample *via* RT-PCR (Bosch Ivalytic, Stuttgart , Germany).

The methaemoglobin determination was performed using the method of Evelyn and Malloy [10], modified by Rodkey [11]. In short summary: The sample was drawn into K2-EDTA tube, and 200 uL of sample was washed with physiological saline (10 mL). Thereafter, the sediment was suspended in distilled water and haemolyzed. The membrane fragments were removed *via* centrifugation.

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Sex	Pat. No.	METHGB%	Tumour	Ventill	Medicine	Transfusion	HGB	RDW
F	1	4.2	-	-	-	No	14.1	13.1
M	2	2.9	-	-	-	No	15.1	14
F	3	4.16	-	-	-	Yes	13	17.4
M	4	2.7	-	-	-	no	17.6	13.1
F	5	0.54	-	-	-	no	13.7	14.2
M	6	0.27	-	-	-	no	15.3	14
F	7	3.9	-	-	-	no	14.2	14.4
M	8	1.6	-	-	-	yes	14	21.1
F	9	0.5	-	-	-	no	13	13.5
F	10	0.7	-	-	Azithromycine	yes	10.4	18.3
F	11	5	-	-	-	no	13.9	13.5
F	12	5	-	-	-	no	12.8	13.2
F	13	2.1	-	APRV	-	yes	7.1	15.6
M	14	1	-	-	-	no	16.3	13.4
F	15	4.5	-	SIMV	Lidocain	yes	7.4	15.7
F	16	10	-	-	-	yes	11.2	16.2
F	17	66.6	-	-	-	yes	6.9	17.4
M	18	25	-	-	-	yes	8	16.6
M	19	90	-	-	-	yes	7.8	19
F	20	31.5	-	-	-	yes	10.6	18
M	21	30	-	-	-	yes	11	20.5
F	22	0	-	-	-	no	13	13
M	23	0.9	-	-	-	no	16.8	14
M	24	62.9	Non-Hodgkin Hodgkin	-	-	yes	7	25.8
F	25	3	-	-	-	no	12.9	12.9
F	26	2.5	-	-	-	no	13.5	13
F	27	12.5	-	-	-	yes	10.6	13.6
F	28	0	-	-	-	yes	12.4	17.6
F	29	3	-	-	-	no	13	13.9
F	30	0	-	-	-	no	15	13.7
F	31	0	-	APRV	Azithromycine	no	13.4	13.2
M	32	3	-	-	-	no	15.8	12.4
F	33	9	-	-	-	yes	12.4	13.5
M	34	0	-	-	-	no	17.6	13.1
M	35	11	-	-	-	yes	10.9	16.8
F	36	2	-	-	-	no	13.4	13.5
F	37	16.5	-	-	Azithromycine	yes	9.7	23.6
M	38	0	-	-	-	no	14.8	13.8
M	39	60	-	-	-	yes	8.7	22.5
F	40	8	-	-	-	no	9.2	15
F	41	8	-	-	-	no	11	15
M	42	9	-	-	-	yes	10	17
F	43	4	-	-	-	no	12.1	13.9
F	44	9	-	-	-	no	12.8	13
F	45	0	-	-	-	no	13.3	14.3
M	46	9	-	-	-	no	9.1	12.8

Table 1: The details data for 46 patients.

The reverse reduction was blocked in a cold-water bath. After supplementing the hemolysate with 0.1 M phosphate buffer (pH=6.9), the haemoglobin was transformed to haemoglobin-cyanide through potassium ferricyanid. The methaemoglobin was transformed to methaemoglobin cyanide using potassium cyanide. Each derivative was evaluated *via* spectrophotometry (gap width 0.1 nm, light path 10 mm, 630 nm, Nano-drop, Thermo-Fisher, Waltham, MA, USA). The percentage of methaemoglobin was calculated using the formula published in Rodkey's paper [11]. All samples were evaluated using a Siemens Advia 2120 (Erlangen, Germany) haematological analyser. CRP and D-dimer were measured in every patient.

Statistical method

The distribution of data was checked *via* an Anderson Darling test. $p<0.05$ was considered to be non-Gaussian distribution. The medians of non-normal data were compared using Wilcoxon-Mann-Whitney method. In case of normal data, the means were compared with two-samples Student t test. Categorial data were analysed using Chi-square

test. The odds ratio of mortality was also computed using binary logistic regression. All statistical computations were conducted in Minitab 19.0.(Pennsylvania, USA). No. of licence by Ethical Committee of Almási Balogh Pál City Hospital Ózd, Hungary: 1/2022.

Results

Here, we show the detailed data for all 46 patients in Table 1. The reproducibility of the method was tested on 20 healthy blood donor samples, and the relative standard deviation (C.V.%) remained below 3% (median 0.89%, maximal value 2%). The methaemoglobin median percentage of all patients was 3.95% (min. 0%, max. 90%, first quartile 0,85%, third quartile 9.25%). The methaemoglobin results were divided into two groups: deceased patients (no. 15) and patients who survived (no. 31). The median of the first group was 8% (first quartile 4.25%, third quartile 42.5%, minimum 0%, maximum 90%). The median of the second group was 2.5% (first quartile 0.385%, third quartile 4.6%, minimum 0%, maximum 31.5%; see The p value of the Wilcoxon test was 0.001. We also checked the difference using the Chi-square test. The arbitrary cut-off value of methaemoglobin reference/pathological was 2%. The patients were evaluated in a 2 × 2 table: those who died and those who survived, along a 2% methaemoglobin cut-off. The Chi-square p value was 0.0054. The RDW median of patients having methaemoglobinemia less than 10% was 14.5. On the other hand, for patients with levels above 10%, the RDW median was 18.8 (p=0.545)

Further, 19 patients received transfusions. Their median methaemoglobin value was 11% (first quartile 4.33%, third quartile 30.75%, minimum 0%, maximum 90%). Conversely, those who did not receive transfusion showed the following values: methaemoglobin median was 2.7%, first quartile 0.385%, third quartile 4.1%, minimum 0%, maximum 9%. The level of significance was p=0.001. The binary logistic regression did not prove an elevated odds ratio for mortality (OR; 1.07) in patients with elevated methaemoglobin. On the other hand, there was a correlation between the fact of transfusion and the risk of death (OR: 3.15).

Among the transfused patients, those who died showed a methaemoglobin median of 25%. The non-transfused patients' median was 8%. The p value of the Wilcoxon test was 0.001. We also checked that the methaemoglobin levels transfusion can predict mortality. In this case (predictor: methaemoglobin), the slope was 0.0688, constant -1.4424. The odds ratio was 1.0713 (CI95% 1.0075-1.139). In the same way, we checked how transfusion was able to predict mortality. The slope was 1.1474, the constant -1.2576, and the odds ratio 3.15 (C.I.95% 0.88-11.31). The correlation between the RDW and methaemoglobin is illustrated in Rsq (adjusted)=38.4%. The RDW and transfusion also showed a difference. The RDW median of non-transfused patients was 13.5 (first quartile 13.1, third quartile 14). The median RDW of transfused patients was 17.4 (first quartile 16.4, third quartile 19.75) (p<0.05).

The CRP values and methaemoglobin % did not show a significant correlation It is worth highlighting two cases (both patients died within 24 hrs). The first case was a 64 y.o. male patient. His methaemoglobin value was 62.9%. Other values were haemoglobin 70 g/L, haematocrit 18%, RDW 28.5, lymphocytes 4.9%, CRP (ultrasensitive) 60 mg/L, and LDH 436 IU/L D dimer 4.9 mg/L. pH=7.48; pCO2=26.6; pO2= 56.5; sO2=90.9%; p50c=23.7 Hgmm.

The second case was a 76 y.o. male patient. His methaemoglobin value was 90%. Other values were haemoglobin 78 g/L, RDW 19, lymphocytes 3.8%, CRP 32.5 mg/L, D-dimer 3.4 mg/L, LDH 464 IU/L, pH= 7.11, pCO 49.6 Hgmm, pO2 72.4 Hgmm, sO2 91.7%, and p50c 25.9 Hgmm. It is worth pointing out that the oxygen saturation was above 90% in both cases. Some papers emphasize the clinically relevant cut-off value of methaemoglobin as 10%. Below 10%, the significance of methaemoglobin is questionable [11]. We accepted this position and, henceforth, we grouped the patients accordingly.

Analysing all the data, we divided the patients into two groups: 35 patients had methaemoglobin levels below 10%. Their RDW mean was 14.5. Further, 11 other patients showed methaemoglobin above 10%. Their RDW mean was 18.8 (SD=3.97. p=0.0525). It is also worth grouping RDW data from another point of view: the mean RDW of the 15 patients who died was 16.9 (SD 3.65); on the other hand, the mean RDW of the 31 survived patients 14.9 (SD 2.746 p=0.01). Additionally, the RDW's means revealed a distinction between individuals who had transfusions and those who did not.

The mean RDW of transfused patients was 18.2 (SD=3.22) vs. non-transfused -13.57 (SD 0.13 p<0.001). The CRP results did not show a significant difference according to the methaemoglobin. The patients with elevated methaemoglobin level (>10%) had a mean CRP 56.4 (SD 58.69) versus the patients with non-elevated patients' mean 30.4 (SD=40.3 p=0.055145).

We performed two binary logistic regression calculations in order to judge the elevated methaemoglobin level as an independent risk factor for mortality. The predictor of methaemoglobin was 0.062, the constant was -1.266, and the odds ratio 1.06. The other binary logistic regression tested transfusion for its effects on mortality. The predictor of transfusion was 1.1474, the constant was -1.2527, and the odds ratio was 3.15.

Discussion

In normal haemoglobin, must be in ferrous sate is 2+. When iron is oxidized to 3+ by different compounds, it is no longer able to transport oxygen. An essential element of the pathomechanism is that the dissociation curve of residual deoxyhaemoglobin is shifted to the left. A shift to the left indicates increased hemoglobin affinity for oxygen and unfortunately an increased reluctance to release oxygen. Thus, the delivery of oxygen to the periphery tissues is diminished [12].

The biochemistry and pathophysiological importance of methaemoglobin have been known for a long time [13]. The pathomechanism includes the exposition of toxic chemicals (anilin dyestuff, salicylate, nitrate), application of anaesthetics, some antibiotics, and anti-malarial drugs. The metabolites of the above-mentioned chemical structures are highly oxidative. Since their electron affinity is greater than that of the prophyrin ring, they are able to take an electron from hemoglobin. The same applies to anesthetics, some antibiotics (azithromycin) and antimalarial drugs.

The genetically determined disposition (glucose-6 phosphate dehydrogenase deficiency) of some nutritional factors (favism) means that there is an elevated risk. Levels above 30% mean clinical symptoms are already present, and greater than 70% means an immediate danger to life. Unfortunately, due to material and sample organization reasons, we could not obtain the glucose6phosphate dehydrogenase reagent.

There are not many publications about the importance of methaemoglobin in relation to COVID-19 [2-4]. It is noteworthy that, in addition to methaemoglobin, carboxyhaemoglobin can also be detected [4]. Some papers emphasize the importance of ruling out the genetically determined deficiency in glucose-6-phosphate dehydrogenase deficiency. This enzyme provides energy to the methaemoglobin

reductase enzyme that constantly reduces the methaemoglobin back to haemoglobin. As a result of this enzyme action, the methaemoglobin level remains below 2% under normal conditions. Its deficiency is an increased risk factor, but the published results are conflicting. [14]

One patient who died had elevated methaemoglobin and low haemoglobin levels, and the left-shifted haemoglobin dissociation curve is noteworthy (p50c=23.7 mmHg). There is no unified position in the literature on this phenomenon. There is publication about left-shifted dissociation curves in COVID-19 patients, but other authors did not confirm this finding that would be a good explanation for methaemoglobin but this phenomenon is also observable in anaemic patients, independent of COVID-19 [15, 16]. This is why the most obvious explanation is anaemia itself, considering the fact that all such patients suffered from acute anaemia.

Another aspect to analyse in our data is the correlation between the red blood cell size distribution in Gaussian distribution (RDW) and the methaemoglobin level. RDW can widen in different pathologic conditions (inflammation, iron-deficiency anaemia, cardiovascular diseases and bone marrow insufficiency after transfusion). We found only one paper publishing a possible significance of RDW in COVID-19 [17]. The authors found a good correlation between the widened distribution of red blood cells and mortality.

In our data, the group with methaemoglobin levels above 10% had a mean RDW of 18.08 (SD 3.97); otherwise, the group had clinically insignificant methaemoglobin levels. Their RDW mean was 14.5 (p=0.0525: not significant). We cannot provide any causal explanation for this widening, but we consider the phenomenon important to mention.

On the other hand, it was interesting to observe the significant difference in RDWs between the patients transfused and the group who did not receive transfusion. Of course, it makes no sense to define an immediate causality. We cannot tell after a while what was more basic: the anaemia and/or the respiratory failure as a reason for transfusion or the transfusion as a cause for higher RDW. The causal relationship between widened RDW and the fact of transfusion has long been known.The reason is the heterogeneity in size between the donor and recipient erythrocytes.

There is currently no satisfactory explanation for the haemoglobin oxidation in COVID-19. Interesting research has been published. A virus peptide was synthesized in vitro based on a DNA sequence that was able to bind to the haemoglobin chain. After coupling, there was an observable peak at 630 nm, which is the characteristic absorption maximum of methaemoglobin [18].

Other papers pointed out the importance of local anaesthetics or azythromycin in haemoglobin oxidation. Our number of cases was too low to draw a clear conclusion [19, 20].

Consistent with other communications, we did not find a prognostic value of supersensitive CRP results). On the other hand, a higher methaemoglobin value tended to be associated with higher CRP levels, but the p value (0.51) did not prove a significant correlation [21, 22].

One paper raised the possibility of accumulation of methemoglobin in (long)-stored blood conserves [23]. The methaemoglobin level may increase over time due to the exhaustion of the methaemoglobin reductase enzyme. Another paper mentioned anevidence for the effect of transfusion in anaemic patients for elevated methaemoglobin levels in stored blood [24].

The standardization of measurement would be a necessary step to move forward. Different published studies were based on different selection biases and different measurement approaches. The spectrophotometry methods use different wavelengths, different means to eliminate interference, and different computational algorithms. Unfortunately, the traceability of point of care devices used in intensive care units to reference laboratory methods is not complete. Applying clinicians are not always aware of this either. This is a borderline area that is often outside the scope of international standardization recommendations. We had neither the goal nor the competence to fill this gap.

Conclusion

The relationship between high methaemoglobin levels and Covid 19 that is a complication which is caused by the disease not by the virus. The severity of the condition is in relation with the high methaemoglobin levels which were measured. The main cause was the ARDS which is associated with the inflammation, edema and severe pulmonary infiltration, leading to impaired alveolar homeostasis, alteration of pulmonary physiology resulting in pulmonary fibrosis, endothelial inflammation and vascular thrombosis. Methaemoglobin which is the oxidized form of haemoglobin does not bind oxygen and as a result cannot deliver oxygen to the tissues. For this very reason, we would not limit the direction of future research to the role of methemoglobin in COVID-19. We think that oxydo-reduction mechanisms disturbed by inflammation are more worthy of study in general.

References

1. von Reinbold B (1913) Beitrag zum Wissen des Methemoglobins . Hoppe-Seyler Z.Physiol Chem 7:1-33.
2. Hina F, Alexi B and Osama Gaber A (2020) Unexplained methemoglobinemia in coronavirus disease 2019: A case report. A A Pract 14:e01287.
3. Naymagon L, Berwick S, Kessler A (2020) The emergence of methemoglobinemia amids tthe COVID-19 pandemic. Amer J Hematol 58:196-197.
4. Scholkmann F, Restin T, Ferrari M (2021) The Role of methemoglobin and carboxyhemoglobin in COVID-19: A Review. J Clin Med 50:1-15.
5. Lopesa DV, Netoa L, Marquesa LC (2021) Methemoglobinemia and hemolytic anemia after COVID-19 infectionwithout identifiable eliciting drug: A case report. J Clin Med 23:e01013.
6. Feiner JR, Bickler PE, Mannheimer PD (2010) Accuracy of methemoglobin detection by pulse co-oximetry during hypoxia. Anesth Analg 111:143-148.
7. J Dötsch, S Demirakça, D Hahn, N Katz, P G Kühl, et al. (1999) Accuracy of methemoglobin measurements: Comparison of six different commercial devices and one manual method. Crit Care Med 27:1191-1194.
8. Hulse E, Shiana F, Buckley N A (2016) Methemoglobin measurements are underestimated by the Radical 7 co-oximeter: Experience from a series of moderate to severe propanil poisonings. Clin Toxicol 54: 826-828.
9. van Kampen EJ, Zijlstra WG (1983) Spectrophotometry of hemoglobin and hemoglobin derivatives. Adv Clin Chem 23:199-253.
10. Evelyn KA, Malloy HT (1938) Microdetermination of oxyhemoglobin, methemoglobin and sulfhemoglobin in a single sample of blood. J Bio l Chem 126:655-664.
11. Rodkey LF, O'Neal JD (1974) Efftecs of carboxyhemoglobin on the determination of methemoglobin in blood. Biochem Med 9:261-270.
12. Wright RO, Lewander J, Wolf AD (1999) Methemoglobinemia: Etiology, pharmacology and clinical management. Review Ann Emerg Med 34:646-656.
13. Jamerson BD, Ho Haryadi C, Bohannon A (2020) Glucose-6-phosphate dehydrogenase deficiency: an actionable risk factor for patients with covid-19? Arch Med Res 51:743-744.

14. Vogel DJ, Formenti F, Retter JA, Vasques F, Camporota L (2020) A left shift in the oxyhemoglobin dissociation curve in patients with severe coronavirus disease. Br J Haematol 191:390-393.

15. Gille T, Sesé L, Aubourg E, Bernaudin JF, Richalet JP, et al (2022) Is there a shift of the oxygen-hemoglobin dissociation curve in COVID-19? Letter to the et al. Am J Physiol Lung Cell Mol Physiol 322:L174-L175.

16. Boning D, Kuebler WM, Bloch W (2021) The oxygen dissociation curve in COVID-19. Am J Physiol Lung Cell Mol Physiol 321: L349-L357.

17. Ramachandran P, Gajendran M, PerisettiA (2022) Red blood cell distribution width in hospitalized COVID-19 patients. Front Med 8: 582403.

18. Lechuga GC, Souza-Silva F, Sacramento CQ, Trugilho MR, Valente RH, et al.(2021) SARS-cov-2 proteins bind to hemoglobin and its metabolites J Mol Sci 22:9035.

19. Martin LO, Gerald MK (1981) Methemoglobinemia induced by local anesthetics. Amer J Hosp Pharmacy 38:89-93.

20. Guay J (2009) Methemoglobinemia related to local anesthetics: A summary of 242 episodes. Anest. Analg 108:837-845.

21. Chen L, Liu HG, Liu W, Liu J, Liu K, et al (2020) Analysis of clinical features of 29 patients with 2019 novel coronavirus pneumonia. Zhonghua Jie He He Hu Xi Za Zhi 43: E005.

22. Wu C, Chen X, Cai Y, Zhou X, Xu S, et al. (2020) Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern.Med 180:934-943.

23. Uchida I, Tashiro C, Koo YH, Mashimo T, Yoshiya I (1990) Carboxyhemoglobin and methemoglobin levels in banked blood. et al. J. Clin. Anest 2: 86-90.

24. Hare GM, Tsui AK, Crawford JH, Patel RP (2013) Is methemoglobin an inert bystander, biomarker or a mediator of oxidative stress-The example of anemia? Redox Biol 1:65-69.