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Life-threatening course in coronavirus disease 2019 (COVID-19): Is there a link to methylenetetrahydrofolic acid reductase (MTHFR) polymorphism and hyperhomocysteinemia?

Matthias Karst[a], Josef Hollenhorst[a], Johannes Achenbach[a,b,*]

[a] Department of Anesthesiology and Intensive Care Medicine, Pain Clinic, Hannover Medical School, Hannover, Germany
[b] Department of Anesthesiology, Intensive Care Medicine, Emergency Medicine and Pain Medicine, Nordstadt Krankenhaus Hannover, Hannover, Germany

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ABSTRACT

As the current COVID-19 pandemic develops and epidemiological data reveals differences in geographical spread as well as risk factors for developing a severe course of illness, hypotheses regarding possible underlying mechanisms need to be developed and tested. In our hypothesis, we explore the rational for a role of MTHFR polymorphism C677T as a possible explanation for differences in geographical and gender distribution in disease severity. We also discuss the role of the resulting hyper-homocysteinemia, its interaction with the C677T polymorphism and its influence on immune state as well as risk factors for severe disease. Finally, we consider possible dietary ways to influence the underlying pathomechanisms prophylactically and supportively.

Originating from China at the end of 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak evolved into a pandemic. Patients with SARS-CoV-2 infection can develop coronavirus disease 2019 (COVID-19), which is associated with an 8.8% mortality rate in the cohort aged 60 years and above in comparison to 0.46% for patients aged below 60 years [1]. Countries with the highest mortality rates are Italy, Spain, Iran, France, and the USA [1]. A very recent report from Italy including 1591 critically ill COVID-19 patients showed that the vast majority were older men [2]. This is consistent with the mortality rate worldwide showing a predilection for the male gender (M:F = 1.7:1) [1] and presence of at least one comorbidity in 68% of cases. Similar to previous reports [1], hypertension was the most common comorbidity, followed by cardiovascular disease, hypercholesterolemia and diabetes [2]. Compared to other western countries, in Italy high rates of ICU admission (9%) and ICU mortality (26%) were observed [2] while the death rate of 12.8% for the whole country appears to be quite high [2]. Sharply increased values of neutrophils and pro-inflammatory cytokines such as IL-6 and TNF-alpha [1], abnormal coagulation tests and disseminated intravascular coagulation are frequently observed in deaths from COVID-19 [3].

The 5,10-methylenetetrahydrofolic acid reductase (MTHFR) enzyme is most important in the one-carbon-methionine pathway regulating fundamental processes in cell physiology such as DNA repair, neurotransmitter functions and membrane transport [4]. The T allele of the MTHFR-gene (C677T polymorphism), in which cytosine is replaced by thymine at the 677th position, has been suggested to be protective against malignancies such as colon cancer and acute lymphatic leukemia [4,5]. This mutation leads to a thermolabile enzyme variant in which the dissociation rate of the cofactor flavin adenine dinucleotide (FAD) is increased reducing its activity to an extent of 50% or more [4,5]. In people possessing a medium skin tone, which evolutionarily developed in Eurasia under moderate UV radiation, the basic MTHFR function is largely preserved as long as there is sufficient dietary folic acid [4,5]. However, with insufficient folate intake the reduced activity of the MTHFR enzyme leads to reduced levels of 5-methyltetrahydrofolate (5-MTHF) and thus reduced activity of methionine synthetase culminating in accumulation of the key metabolite homocysteine (Hc) to toxic levels [4]. MTHFR C677T polymorphism is the most common MTHFR single nucleotide polymorphism (SNP) and the most common genetic cause of hyper-homocysteinemia (H-Hcy) [4]. The global prevalence of both the CT and TT genotype was found to be highest in Europeans (54,0%) and North Americans (42,8%) and lowest in Asians (35,4%) and Africans (19,6%) [5]. However, subgroup analysis showed remarkable regional differences. Among East Asian countries, both genotypes were found to be most prevalent in China (67,1%) and least prevalent in India (20,3%). In European countries the highest prevalence was found in Italy (66,3%), the lowest in Finland (44,2%) [5,6]. While the TT genotype was found significantly more often in males of the Indian cohort [5], generally, the C677T polymorphism seems to be distributed equally between genders [7]. However, low folate status

* Corresponding author.
E-mail address: johannes.achenbach@googlemail.com (J. Achenbach).

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resulted in significant higher levels of Hc only in male subjects [7,8]. Further, the C677T mutation seems to be associated with a significantly increased risk for coronary artery disease only in homozygous men [9].

Regardless of the folate plasma level, other risk factors for the development of H-Hcy are chronic renal failure, hypothyroidism and malignant tumors of the breast, ovary, and pancreas [10]. Furthermore, adverse lifestyle factors such as smoking, alcohol consumption, and physical inactivity can elevate Hcy levels [11]. Additionally, H-Hcy is more often found in the elderly and in men [10] which may in part be caused by lower serum levels of folic acid and vitamin B12, reduced methionine metabolism, and higher serum creatinine levels in men compared to women [12].

H-Hcy is associated with the development of atherosclerotic vascular disease including arterial hypertension and congestive heart failure [10]. The underlying mechanisms are nitric oxide (NO) antagonism, production of reactive oxygen species (ROS), pro-thromboplastic activity, loss of blood vessel vasorelaxation and alterations in the elastin/collagen ratio [10]. Further, there is a correlation between H-Hcy and arterial hypertension that especially applies to males as androgen hormones, in contrast to estrogen, increase ACE and angiotensin-receptor-1 (AT1R) activity [13,14]. Altogether, these changes result in vascular cell dysplasia, endothelial dysfunction and a procoagulant state [10].

While these processes are slow and develop over years, acute H-Hcy activates a pro-inflammatory cascade through upregulation of the nuclear transcription factor (NF-kB) in neutrophils and macrophages, which release an ample amount of ROS potentiating oxidative stress [11]. This acute H-Hcy can be triggered independently of folate status, when a systemic inflammatory process develops [11]. The increased production of ROS through an acute respiratory viral infection additionally overwhelms the oxidant defense system. The ROS-activated NF-kB accelerates viral replication, which has been previously shown in SARS CoV-1 infection [15]. Interestingly, in line with these findings COVID-19 patients' plasma homocysteine levels showed a predictive value for progression of pathological findings in chest CT-imaging [16]. In contrast to the usual definition of H-Hcy with values above 15 μmol/L the cutoff-value of Hcy predicting imaging progression was 10.58 μmol/L [16].

There is also a relationship of MTHFR polymorphisms and reduced levels of glutathione as the folate cycle, the methionine cycle and the transsulfuration pathway are intricably linked [17]. S-adenosyl-methionine levels are lowered in states of low MTHFR activity which results in decreased stimulation of cystathionine beta-synthase (CBS), the enzyme that shuttles homocysteine into the transsulfuration pathway that ultimately leads to the synthesis of glutathione [17]. In an interesting case report, the therapeutic supplementation of glutathione lead to rapid symptom improvement of two cases of Covid-19 all of which points to the well-known role of glutathione as an important part of the anti-oxidative defense system in viral illness [18]. Interestingly, methylome-wide association analyses identified 13 probes significantly associated with the interaction of mild H-Hcy and C677T polymorphism [19]. The most significant associations were observed with a cluster of probes at the angiotensin II receptor associate protein-methylenetetrahydrofolate reductase-natriuretic peptide A/B (AGTRAP-MTHFR-NPPA/B) gene cluster on chromosome 1 [19]. As SARS-CoV-2 enters and infects cells through angiotensin II receptors these changes in DNA-methylation may result in an increased and specific vulnerability to SARS-CoV-2. In addition, differential methylation at that region on chromosome 1 is functionally associated with variability in expression of the *TNFRSF8* gene [19]. As a member of the TNF-receptor superfamily, this gene encodes a protein (CD30) that mediates a signal transduction pathway leading to NF-kB activation [19]. Therefore, C677T-Hc-interaction may result in an increased activation of NF-kB promoting production of ROS and viral replication as outlined above.

What are the consequences?

In addition to the MUST- and NRS-2002 criteria evaluating nutritional risks in polymorbid individuals [20], subjects at high risk for adverse outcomes when infected with COVID-19, such as the elderly with comorbidities, should be screened for H-Hcy.

Concentrations above 8 μmol/L should lead to the implementation of improvements in diet quality (fruit, vegetables, whole grains, fresh meats, and seafood) [21] and the additional application of 5-MTHF (the most biologically active form of folic acid) which bypasses MTHFR ± B-vitamins [22]. Supplementation of folic acid alone, especially in countries practicing dietary folic acid fortification, can even lead to the opposite effect in regular subjects and even more pronounced in patients with MTHFR polymorphism. The proposed mechanism is an accumulation of unmetabolized folic acid which inhibits MTHFR through excess-substrate inhibition and binding competition with 5-MTHF [22]. As co-factors of enzymes in Hcy metabolism the vitamins B6 [21] and B12 [10] and B2 (riboflavin) (a precursor of FAD which stabilizes MTHFR) should be added [23–25]. Vitamin B6 and riboflavin have been previously shown to lower Hcy levels [24,26].

To test the hypothesis of dietary down-regulation of Hcy levels to prevent poor outcomes in Covid-19, a prospective controlled trial including genetic testing for MTHFR polymorphism could be helpful. Such an interventional study is particularly important since supplementation of synthetic folic acid and vitamins in H-Hcy and cardiovascular disease showed mixed and modest preventative effects on final outcomes so far [10,11].

In active COVID-19 disease, the determination of H-Hcy through serum levels as opposed to genetic testing for MTHFR polymorphism may be used as an early biomarker for adverse outcomes and higher mortality and may serve as indication for vigorous supportive nutritional interventions. In addition to B vitamins, vitamins A, C, D, and E, omega-3 polyunsaturated fatty acids (PUFA), selenium, zinc and iron have been shown to possess direct antiviral properties [27]. As diet-derived antioxidants make the most significant contribution to the body's antioxidant defense system [28], the administration of strong antioxidants, i.e. Vitamin C, may help to prevent both, further tissue damage and increased viral replication. In 2003, Vitamin C was already proposed as a treatment option in the SARS CoV-1 outbreak [29]. In COVID-19 a randomized controlled trial with 12 g Vitamin C administered twice daily IV has been started in China [30].

In conclusion, we propose a theory of specific vulnerability to a severe course of COVID-19 initiated by H-Hcy, which can be triggered by the presence of the C677T polymorphism. Male gender, nutritional factors, life-style factors and several underlying diseases seem to be further significant risk factors for an increased vulnerability to SARS-CoV-2. During the SARS-CoV-2 pandemic early risk stratification by measurement of Hc-plasma levels and possibly screening for the presence MTHFR polymorphism appears promising. Additionally, treatment with vitamins and micronutrients in addition to standard supportive care seems to be warranted to protect and support the most vulnerable patient groups.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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this manuscript has not been previously published and is not currently under consideration by any other journal. Additionally, all of the authors have approved the contents of this paper and affirm that this manuscript is an honest, accurate and transparent account of the topic being reported; no important aspects have been omitted. They have agreed to Medical Hypotheses' submission policies. MK devised the initial concept of the paper and MK and JA were involved in drafting, writing and revising the manuscript as well as the literature survey. JH contributed to arrangement, proof-reading, critical review and the literature survey.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2020.110234.

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