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Perspective

Enhanced vitamin K expenditure as a major contributor to vitamin K deficiency in COVID-19

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ABSTRACT

Objectives: Vitamin K deficiency consistently associates with worse clinical outcome in COVID-19 patients. However, whether this is due to increased expenditure during inflammation or poor vitamin K status prior to infection remained unknown.
Methods: Dp-ucMGP levels of 128 individuals were measured for the post-MONICA study and were compared to SARS-CoV-2 PCR testing results.
Results: Dp-ucMGP levels prior to COVID-19 infection were not significantly different comparing PCR-negative, PCR-positive and not hospitalized, and PCR-positive and hospitalized patients.
Conclusion: In this study, we demonstrate normal vitamin K status prior to infection in SARS-CoV-2 positive patients, supporting the theory of increased utilisation during disease.

Introduction

In December 2019, the acute respiratory disease COVID-19, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), made its appearance in Wuhan, China (Huang *et al.*, 2020). Several studies trying to clarify the COVID-19 pathogenesis or investigating different remedies have been conducted and reviewed (Oliaei *et al.*, 2021; SeyedAlinaghi *et al.*, 2021); however, the need to elucidate the disease pathways of this poignant disease remains. Severe vitamin K deficiency has been demonstrated in hospitalized COVID-19 patients and consistently been associated with worse clinical outcome (Desai *et al.*, 2021; Dofferhoff *et al.*, 2021). Correlations between low vitamin K status and markers of both extracellular matrix elastic fiber degeneration and inflammation suggest an underlying link rather than an epiphenomenon (Desai *et al.*, 2021; Visser *et al.*, 2021). Vitamin K is essential for the carboxylation of coagulation factors in the liver, but it is also crucial as a cofactor for the activation of certain extrahepatic proteins. Matrix Gla protein (MGP) is a vitamin K-dependent protein that protects soft tissue against calcification (Schurgers *et al.*, 2013). Circulating inactive MGP (*i.e.*, desphospho-uncarboxylated MGP [dp-ucMGP]) has been established as a robust biomarker of extrahepatic vitamin K status (Schurgers *et al.*, 2013). A high level of dp-ucMGP reflects a low vitamin K status and *vice versa.* Vitamin K status depends on daily vitamin K intake, in combination with vitamin K usage in our body. Limited variability of dp-ucMGP levels was found in healthy individuals over a time frame of 1 year (Vermeer and Vik, 2020), suggesting that the intake and expenditure normally remain rather stable. Vitamin K deficiency at baseline is associated with disease progression and increased mortality of several conditions, such as cardiovascular, pulmonary, and renal diseases (Wei *et al.*, 2019). Low average daily vitamin K consumption or vitamin K depletion due to a chronic illness may be the cause of elevated dp-ucMGP levels in hospitalized patients with COVID-19 and thus form a risk factor for infection or disease severity. Alternatively, inflammation-induced vitamin K expenditure during the infection may also be the main cause of poor vitamin K status in COVID-19 patients (Janssen *et al.*, 2021). To evaluate the importance of both mechanisms, we compared dp-ucMGP levels of a historical cohort from 7 to 14 years before the emergence of COVID-19.

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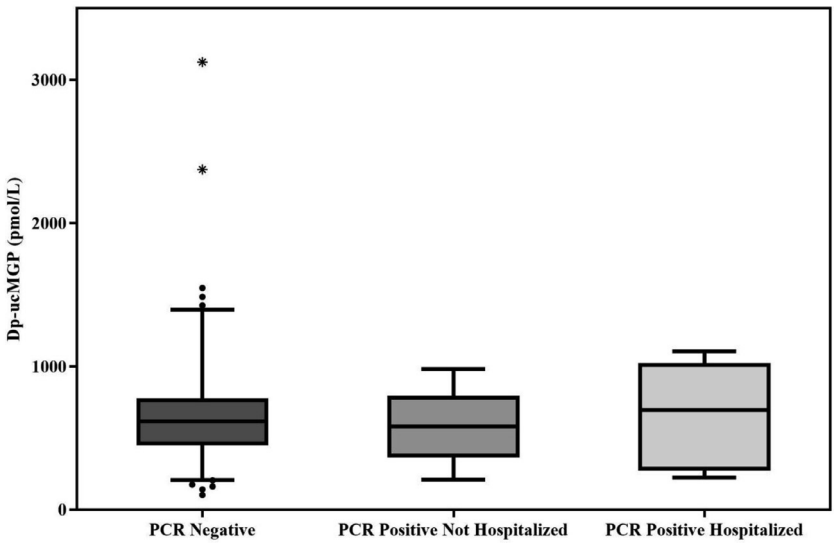

Figure 1. Boxplots (5th percentile, 1st quartile, median, 3rd quartile, and 95th percentile) showing no significant difference in dp-ucMGP levels between PCR negative (n = 104), PCR-positive not hospitalized (n = 8) and PCR-positive hospitalized patients (n = 10). Asterisks indicate extreme outliers.
dp-ucMGP =desphospho-uncarboxylated MGP; PCR = polymerase chain reaction.

Methods

In a Czech cohort of 2651 individuals, dp-ucMGP levels were measured for the post-MONICA study (MONItoring of trends and determinants in CArdiovascular disease) between 2006 and 2013. Details of the post-MONICA study have been described previously (Cífková *et al.*, 2020). In 2020, 128 individuals in this cohort were tested for SARS-CoV-2 using a polymerase chain reaction (PCR) test. Patient data were extracted from hospital records. Dp-ucMGP levels were measured as previously described (Mayer *et al.*, 2016). Individuals were categorized as (i) PCR-negative, (ii) PCR-positive and not hospitalized, and (iii) PCR-positive and hospitalized. Subjects were tested when they had one or more symptoms of coughing, runny nose, sneezing, shortness of breath, elevated temperature, or sudden loss of smell and taste. Individuals using vitamin K antagonists (VKAs) as anticoagulation drugs were excluded from the analysis. This secondary analysis of patients participating in the post-MONICA study was approved by the ethics committee of the University Hospital in Pilsen, Czech Republic on June 4, 2020. All participants signed written informed consent at the time of the first clinical examination, including the consent to future analyses.

Statistics

Statistical analyses were performed using SPSS (version 21, IBM, Chicago, IL, USA). A $P < 0.05$ was used as the threshold for statistical significance, with a confidence interval (CI) of 95%. The ratio of the means was provided as well. Dp-ucMGP levels had a log-normal distribution and were log-transformed before analyses. One-way analysis of variance was used to compare dp-ucMGP levels between two groups. Analysis of covariance was used to assess differences in the mean dp-ucMGP between all groups. In both analyses, we adjusted for sex, age, and body mass index. The means and 95% CIs of log-transformed dp-ucMGP levels were back-transformed into geometric means and 95% CIs.

Results

Of 128 subjects, 19 individuals had a positive PCR test for SARS-CoV-2, and 109 individuals were negative. Of 19 PCR-positive individuals, 11 subjects were admitted to the hospital. Six VKA users were excluded before further analysis (five individuals who tested negative and one hospitalized patient). The mean age was 61 years, and 58.2% were male at the time of inclusion in the post-MONICA study. A total of 59 out of 122 patients had a history of cardiovascular disease. The mean time between the dp-ucMGP quantification and SARS-CoV-2 PCR was 11.7 years.

Dp-ucMGP levels were not significantly different in individuals with a positive PCR test (n = 18, 550.5 pmol/l, 95% CI 419.6-722.3) compared with those with negative test results (n = 104, 579.3 pmol/l, 95% CI 522.5-642.2; *P*-value = 0.855). The ratio of the means was 1.1 (95% CI 0.8-1.5). Dp-ucMGP levels were not significantly different between hospitalized (n = 10, 569.0 pmol/l, 95% CI 368.9-877.7) and non-hospitalized patients (n = 8, 528.3 pmol/l, 95% CI 348.1-801.7; *P*-value = 0.178). The ratio of the means was 1.0 (95% CI 0.5-2.0).

Dp-ucMGP levels were not significantly different comparing PCR-negative, PCR-positive but non-hospitalized individuals, and hospitalized patients (*P*-value = 0.806) (Figure 1). The ratio of the means was 1.0 (95% CI 0.7-1.6).

Discussion

High dp-ucMGP levels have consistently been found in COVID-19 (Dofferhoff *et al.*, 2021; Linneberg *et al.*, 2021). However, we found no difference in dp-ucMGP levels—measured several years before the start of the pandemic—between individuals with positive or negative SARS-CoV-2 PCR in the first period after the outbreak in the Czech Republic. Furthermore, dp-ucMGP levels did not differ between individuals who tested positive and who were hospitalized or remained ambulant. These data support the hypothesis that enhanced vitamin K use during the infection may be the most important reason for vitamin K deficiency in patients with COVID-19.

We previously proposed a mechanism for how SARS-CoV-2 infection may lead to vitamin K depletion (Janssen *et al.*, 2021). Pulmonary cells respond to viral contamination by upregulation of cytokines synthesis, leading to pneumonic infiltrates (Janssen *et al.*, 2021). Attracted immune cells produce proteases, degrading matrix components in the lungs. MGP production is subsequently upregulated as an autologous defense mechanism to prevent permanent damage (Janssen *et al.*, 2021). However, MGP requires vitamin K-

dependent activation for functionality, which may deplete vitamin K stores and induce deficiency.

Furthermore, recent observations of individuals using VKAs support our hypothesis that it is mainly increased vitamin K use during the infection rather than poor baseline vitamin K status responsible for the extrahepatic vitamin K deficiency in our studies (Irwin *et al.*, 2022; Speed *et al.*, 2020). An increase in high International Normalized Ratio (INR) was found in patients of an anticoagulation clinic in south London, within the epicentre of the first COVID-19 outbreak in 2020 (Speed *et al.*, 2020); furthermore, decreased warfarin dose requirements were evident in hospitalized patients with COVID-19, with a mean percentage decrease in dose of 68.8% (Irwin *et al.*, 2022). Overall, these findings are evocative of increased vitamin K use during COVID-19 disease.

Our findings form a rationale to assess the effect of vitamin K supplementation in intervention trials as soon as possible after the diagnosis of SAR- CoV-2 infection. Another strategy that might be explored is the preventive use of vitamin K to obtain adequate stores to compensate for the accelerated use during infection.

Limitations

The baseline determination of dp-ucMGP levels was at least 7 years before disease outbreak. However, it has been demonstrated that dp-ucMGP levels are rather stable over time. Nevertheless, it is a limitation that a second dp-ucMGP measurement was not performed in parallel with the PCR test. Furthermore, the number of hospitalized patients and medical history data were limited, and confirmation in larger cohorts is therefore needed.

Author contributions

CV developed the theory behind stable dp-ucMGP levels over time. RJ developed the theory behind vitamin K in COVID-19. SB and OM collected the data. MPJV analysed the data and wrote the first draft of the manuscript. JW and RJ critically revised the manuscript. All authors read the final manuscript.

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Ethical approval

This secondary analysis of patients participating in the post-MONICA study was approved by the ethics committee of the University Hospital in Pilsen, Czech Republic on June 4, 2020. All participants signed informed consent at the time of first clinical examination, including consent to future analyses.

Declaration of competing interest

RJ declares application of a patent on vitamin K in COVID-19.

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