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Reduced Vitamin K Status as a Potentially Modifiable Risk Factor of Severe Coronavirus Disease 2019

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Background. Respiratory failure and thromboembolism are frequent in severe acute respiratory syndrome coronavirus 2–infected patients. Vitamin K activates both hepatic coagulation factors and extrahepatic endothelial anticoagulant protein S, required for thrombosis prevention. In times of vitamin K insufficiency, hepatic procoagulant factors are preferentially activated over extrahepatic proteins. Vitamin K also activates matrix Gla protein (MGP), which protects against pulmonary and vascular elastic fiber damage. We hypothesized that vitamin K may be implicated in coronavirus disease 2019 (COVID-19), linking pulmonary and thromboembolic disease.

Methods. A total of 135 hospitalized COVID-19 patients were compared with 184 historic controls. Inactive vitamin K–dependent MGP (desphospho-uncarboxylated [dp-uc] MGP) and prothrombin (PIVKA-II) were measured inversely related to extrahepatic and hepatic vitamin K status, respectively. Desmosine was measured to quantify the rate of elastic fiber degradation. Arterial calcification severity was assessed using computed tomography.

Results. dp-ucMGP was elevated in COVID-19 patients compared with controls ($P < .001$), with even higher dp-ucMGP in patients with poor outcomes ($P < .001$). PIVKA-II was normal in 82.1% of patients. dp-ucMGP was correlated with desmosine ($P < .001$) and with coronary artery ($P = .002$) and thoracic aortic ($P < .001$) calcification scores.

Conclusions. dp-ucMGP was severely increased in COVID-19 patients, indicating extrahepatic vitamin K insufficiency, which was related to poor outcome; hepatic procoagulant factor II remained unaffected. These data suggest pneumonia-induced extrahepatic vitamin K depletion leading to accelerated elastic fiber damage and thrombosis in severe COVID-19 due to impaired activation of MGP and endothelial protein S, respectively.

Keywords. COVID-19; elastic fibers; factor II; matrix Gla protein; vitamin K.

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. The majority of individuals who contract SARS-CoV-2 have mild symptoms; however, a significant proportion develop respiratory failure due to pneumonia [1]. COVID-19 may also have extrapulmonary manifestations, including coagulopathy and venous thromboembolism, which are associated with decreased survival [2]. The mechanisms that activate coagulation in COVID-19 remain incompletely understood.

Coagulation is an intricate balance between clot promoting and dissolving processes in which vitamin K plays a well-known role. Procoagulant factor II (FII; ie, prothrombin) requires vitamin K–dependent carboxylation to fulfill its primary function. Vitamin K is also a cofactor of anticoagulant protein S. In contrast to FII, a significant proportion of protein S is extrahepatically synthesized in endothelial cells, which play a local suppressive role against thrombosis [3]. Vitamin K deficiency results in more severely compromised carboxylation of extrahepatic than of hepatic vitamin K–dependent proteins (Figure 1) [4]. This can paradoxically lead to enhanced thrombogenicity in a state of low vitamin K [5].

Matrix Gla protein (MGP) is also vitamin K–dependent but not involved in coagulation [6]. MGP is well known as a calcification inhibitor in arterial walls [7], and MGP's role in the pulmonary compartment seems to be comparable [8, 9]. Elastic fibers are essential matrix components in lungs and have high calcium affinity [10]. Degradation and mineralization of elastic fibers are interrelated processes [11, 12]. Matrix

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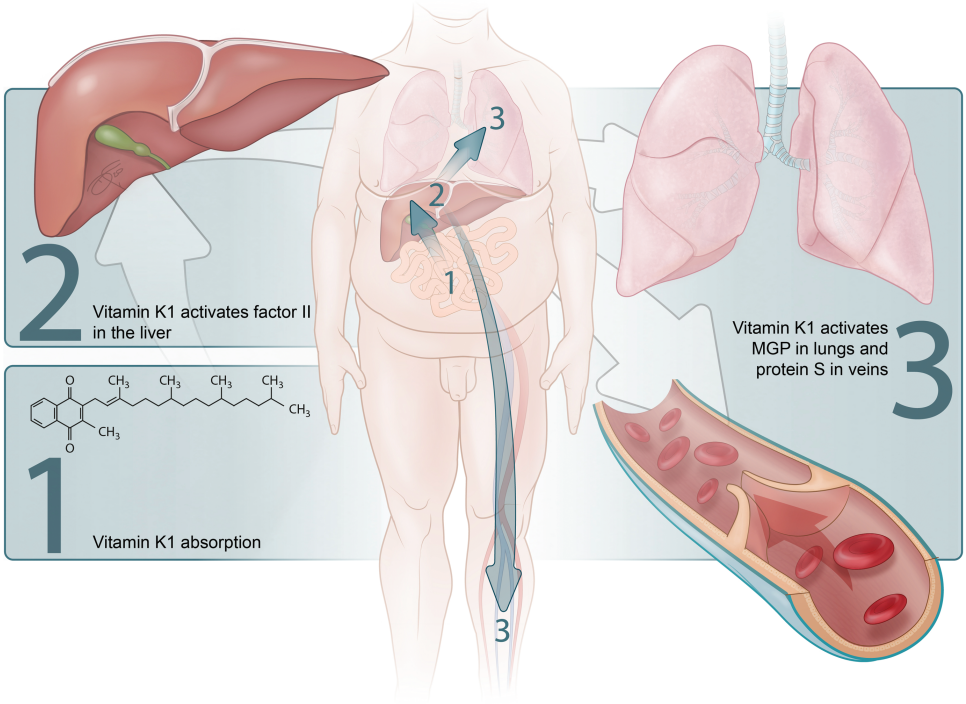

Figure 1. Distribution of vitamin K1 in the body. (1) After absorption, vitamin K1 is preferentially transported to the liver via the portal circulation, where it is used for carboxylation of hepatic coagulation factors. This implies that during periods of vitamin K insufficiency, (2) the grade of carboxylation is usually higher for hepatic factor II (3) than for endothelial protein S in veins and pulmonary MGP. Abbreviation: MGP, matrix Gla protein.

metalloproteinase (MMP) synthesis increases parallel with elastic fiber calcification [13], and partially degraded elastic fibers become prone to mineralization [10]. Recent data show that a subset of MMP-producing macrophages is increased in severe SARS-CoV-2 pneumonia [14]. COVID-19 may theoretically be linked to both vitamin K deficiency and elastic fiber metabolism through a series of sequential pathologic steps (Figure 2).

Individuals with severe SARS-CoV-2 infections often have comorbidities that are associated with reduced vitamin K status, such as hypertension, diabetes, and cardiovascular diseases [1, 7]. The body uses vitamin K very efficiently, and storage capacity is low [15]. There are reasons to suspect that there is increased use of vitamin K for carboxylation of pulmonary MGP and coagulant factors during COVID-19 [16]. Vitamin K depletion may have devastating consequences in the lungs [17].

Our aim in this study was to determine whether a reduced vitamin K status plays a role in the pathogenesis of COVID-19 by interacting with both elastic fiber metabolism and the coagulation cascade, thereby linking pulmonary and coagulopathic disease manifestations.

METHODS

Patients

A total of 135 patients hospitalized with COVID-19 at the Canisius-Wilhelmina Hospital between 12 March 2020 and 15 April 2020 were included. SARS-CoV-2 infection was confirmed using real-time polymerase chain reaction testing. Patient data were extracted from hospital records, and vitamin K antagonist (VKA) usage was determined from pharmacy and anticoagulant clinic records. The United Medical Research Ethics Committees of Canisius-Wilhelmina Hospital approved the study and waived the need for written informed consent. Patients could opt out after they were informed about the study.

A total of 186 controls from a previously published chronic obstructive pulmonary disease (COPD) study were also included [18]. Two control patients for whom the use of VKA was unknown were excluded from the analysis.

Patients were followed up until discharge from the hospital, intubation and mechanical ventilation, or death. The outcome of COVID-19 patients was categorized as "good" if they were discharged from the hospital without the need for invasive ventilation and "poor" if they required either intubation and mechanical ventilation or died. During admission, blood was sampled 3 times per week and EDTA plasma and serum were frozen at −80°C for retrospective analysis.

Desphospho-Uncarboxylated MGP

Direct quantification of blood vitamin K is not appropriate to assess vitamin K status due to differences in bioavailability and half-life time between the 2 naturally occurring forms (vitamin K1 and K2). Additionally, the intake of vitamin K2

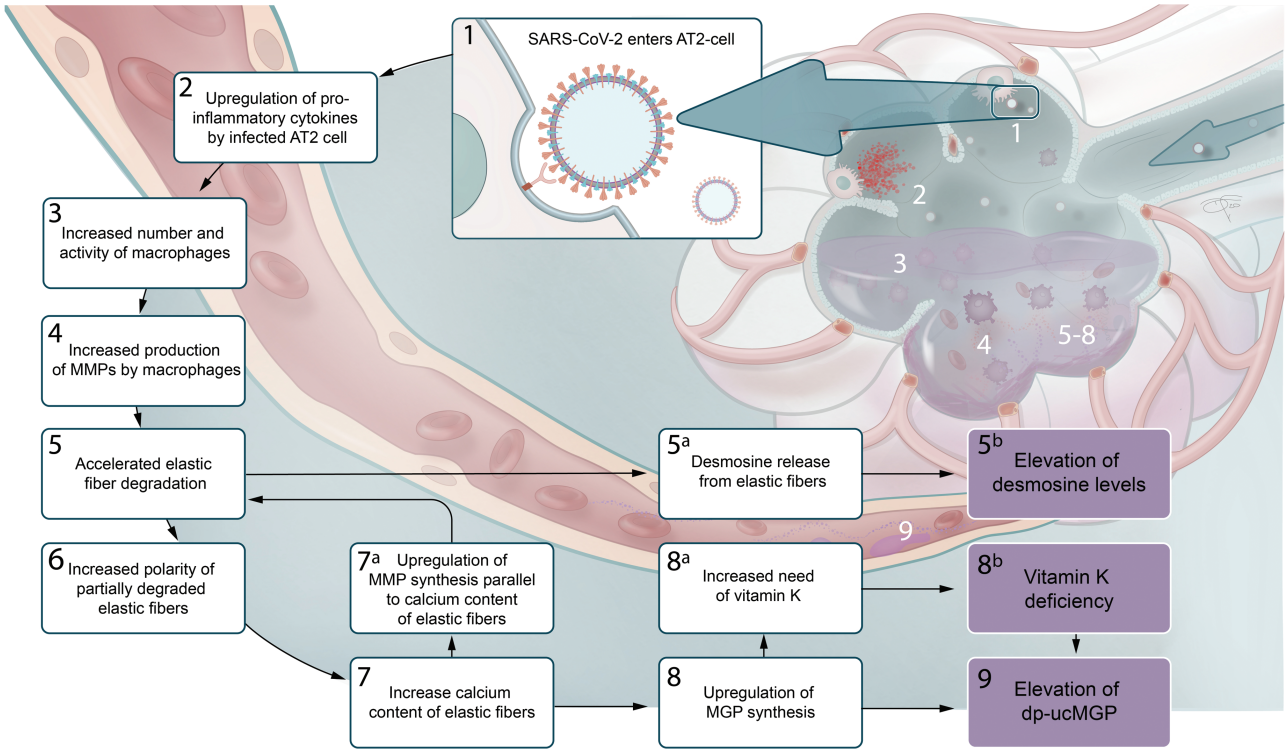

Figure 2. Proposed sequential pathologic steps linking SARS-CoV-2 pneumonia to vitamin K insufficiency and accelerated elastic fiber degradation. (1) SARS-CoV-2 enters AT2 cell. (2) The infected AT2 cell responds by upregulating synthesis of proinflammatory cytokines. (3) This leads to an increase in the number and activation of pulmonary macrophages. (4) These infiltrating macrophages produce MMPs (5), which leads to accelerated degradation of elastic fibers (5a) and thereby the release of desmosine from these fibers (5b), leading to elevated desmosine levels in lungs and blood. (6) The increased polarity of partially degraded elastic fibers (7) enhances their affinity for calcium and, consequently, leads to increased elastic fiber calcium content. (7a) MMP synthesis is upregulated in parallel with the calcium content, which further accelerates elastic fiber degradation in a self-propagating vicious circle. (8) MGP synthesis is upregulated in an attempt to protect elastic fibers from calcification and degradation, (8a) which means that need for vitamin K to activate additional MGP increases. (8b) This increased use of vitamin K may induce vitamin K insufficiency, (9) in which case increased production of MGP in a state of vitamin K insufficiency leads to increased dp-ucMGP in lungs and blood. Abbreviations: AT2, alveolar type II; dp-uc, desphospho-uncarboxylated; MGP, matrix Gla protein; MMP, matrix metalloproteinase; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

is too low to measure accurately. Measuring inactive levels of vitamin K–dependent protein in the circulation is a valuable method to quantify the combined deficit of vitamin K1 and K2. Desphospho-uncarboxylated (dp-uc) MGP (inactive MGP) is an appropriate indirect marker of extrahepatic vitamin K status [19, 20]. Individuals with high dp-ucMGP levels have low extrahepatic vitamin K status and vice versa.

Circulating dp-ucMGP levels were determined in EDTA plasma using the commercially available IVD chemiluminescent InaKif MGP assay on the IDS-iSYS system (IDS, Boldon, United Kingdom) as previously described [21]. The within-run and total precision of this assay were .8%–6.2% and 3.0%–8.2%, respectively. The assay measuring range is between 200 and 12 000 pmol/L and was found to be linear up to 11 651 pmol/L. dp-ucMGP values <300 pmol/L are in the normal healthy range and values >500 pmol/L reflect vitamin K deficiency [22].

Protein Induced by Vitamin K Absence-II

Protein induced by vitamin K absence (PIVKA)-II was used to assess hepatic vitamin K status. Individuals with high PIVKA-II levels have low hepatic vitamin K status and vice versa. Circulating PIVKA-II levels were measured in serum using a conformation-specific monoclonal antibody in an enzyme-linked immunosorbent assay as previously described [23]. The detection limit as well as upper limit of normal was .15 AU/mL [23].

Desmosine

Plasma (p) desmosine and isodesmosine (DES) levels were used as a marker for the rate of elastic fiber degradation [24]. DES are formed during the cross-linking of tropo-elastin polymers and are released in the bloodstream after degradation of elastic fibers [24]. pDES directly reflects the rate of systemic elastic fiber degradation.

DES fractions were measured using liquid chromatography-tandem mass spectrometry as previously described [18, 24]. Coefficients of variation of intra- and interassay imprecision were <8.2%, the lower limit of quantification was 140 ng/L, and assay linearity was up to 210 000 ng/L.

Computed Tomography Assessment

Thin-slice computed tomography (CT) scans were acquired using a Philips Ingenuity multidetector row scanner (Philips

Healthcare). CT images of 1-mm thickness were reconstructed using iterative model-based reconstruction in the axial plane.

Quantitative measurements of the volume of ground glass and consolidation were undertaken using the Intellispace Portal (COPD package, Intellispace version 10, Philips Healthcare). In the software, first the lungs were segmented from the chest wall and major vessels and main bronchi. Manual adjustments were made where required by a board-certified chest radiologist. Subsequently, the lung voxels were counted to derive a total lung volume in milliliters. Diseased lung tissue was defined as those voxels with an attenuation of Hounsfield units (HU) greater than −700 as previously defined. The abnormal voxels were expressed as a percentage diseased lung of the total volume. An HU value at the 85th percentile was used [25, 26].

Coronary aortic calcification (CAC) and thoracic aortic calcification (TAC) were also quantified in the Intellispace Portal (Heartbeat CS package). Calcifications were defined as areas with an HU of 130 and higher. The calcifications were visually localized up to the arterial wall by a board-certified chest radiologist and semiautomatically segmented. The volume was used as a measure of calcification burden.

Statistical Analyses

Statistical analyses were performed using SPSS (version 24, IBM, Chicago, IL). Analysis of variance was used to compare dp-ucMGP, pDES, and radiological scores between groups. Analysis of covariance was used to perform the aforementioned analyses: dp-ucMGP, CAC, and TAC adjusted for age, sex, and use of VKA and pDES adjusted for age.

For each pDES measurement in a COVID-19 patient, virtual age-matched pDES values were calculated using published pDES equations for never and (ex-) smokers [24]. pDES is strongly dialyzed (R. Janssen, unpublished data), and patients receiving dialysis at baseline were excluded from pDES analyses.

The Spearman correlation coefficient was used to test the association of closest time-matched dp-ucMGP with pDES and radiological scores.

For PIVKA-II, patients were categorized as follows: normal <.15 AU/mL, mildly elevated .15–.5 AU/mL, moderately elevated .5–2.0 AU/mL, and severely elevated >2.0 AU/mL.

dp-ucMGP, pDES, and radiological scores had a log-normal distribution and were therefore natural log-transformed prior to analyses. Since CAC and TAC scores included values equal to zero, these values were transformed using Ln(CAC + 1) and Ln(TAC + 1), respectively. The mean difference and 95% confidence interval (CI) of log-transformed values was backtransformed to the mean fold change. Normally distributed continuous variables are presented as mean ± standard deviation, whereas continuous variables with a natural-log distribution are presented as backtransformed mean and 95% CI. A P value of < .05 was used as the threshold for statistical significance.

RESULTS

The mean age of COVID-19 patients was 68 ± 12 years, 93 (69%) were male, and 12 (8.9%) used VKA. Of the historical controls, 85 (46%) were male, 3 (1.6%) were taking VKA, and the mean age was 61 ± 6.5 years. Patient and control characteristics are shown in Table 1.

dp-ucMGP

dp-ucMGP was measured in all available samples. Maximum dp-ucMGP levels were significantly higher in COVID-19 patients (1476 pmol/L; 95% CI, 1341 to 1625) compared with healthy controls (471 pmol/L; 95% CI, 434 to 511; mean fold change, 3.14; 95% CI, 2.76 to 3.56; P < .001; Figure 3A), which remained significant after adjustment for age, sex, and use of VKAs (P < .001). dp-ucMGP levels were significantly higher in

Table 1. Baseline Characteristics of Coronavirus Disease 2019 Patient and Healthy Control Cohorts

	Coronavirus Disease 2019			Controls
Characteristic	Good Outcome, n (%)	Poor Outcome, n (%)	All, N (%)	
Patients	75	60	135	184
Age, y	64 ± 13	72 ± 10	68 ± 12	61 ± 6.5
Male (%)	46 (61)	47 (78)	93 (69)	85 (46)
Vitamin K antagonist use (%)	5 (6.7)	7 (12)	12 (8.9)	3 (1·6)
Hypertension (%)	28 (37)	21 (35)	49 (36)	41 (22)
Diabetes mellitus (%)	15 (20)	15 (25)	30 (22)	14 (7.6)
Cardiac or cardiovascular disease (%)	17 (23)	21 (35)	38 (28)	12 (6.5)
Asthma/Chronic obstructive pulmonary disease (%)	13 (17)	12 (20)	25 (19)	7 (3.8)
Other respiratory disease (%)	5 (6.7)	10 (17)	15 (11)	3 (1.6)
Immunocompromised (%)	4 (5.3)	2 (3.3)	6 (4.4)	0 (0)
Dialysis dependent (%)[a]	1 (1.3)	2 (3.3)	3 (2.2)	0 (0)
Active malignancy (%)	6 (8.0)	6 (10)	12 (8.9)	0 (0)

[a]At admission.

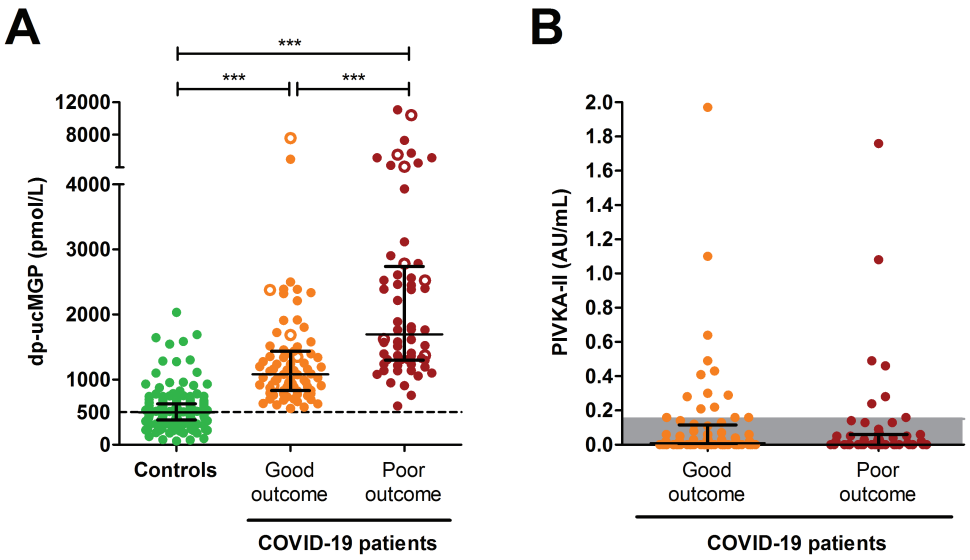

Figure 3. Circulating dp-ucMGP and PIVKA-II in COVID-19 patients. *A*, dp-ucMGP was measured in plasma of COVID-19 patients with a good outcome (discharge without invasive ventilation, n = 75; orange) or poor outcome (invasive ventilation and/or death, n = 60; red) compared with a cohort of controls. Patients with high dp-ucMGP have low extrahepatic vitamin K status and vice versa. The maximum dp-ucMGP measured during the study is shown, with open circles representing those patients using vitamin K antagonist (VKA) at admission. *B*, PIVKA-II was measured in plasma at baseline in those patients not using VKA (n = 122). The detection threshold and normal range for healthy controls is shown in gray. A single patient not using VKAs had a severely elevated PIVKA-II outside the detection range and is not shown in the figure. Abbreviations: COVID-19, coronavirus disease 2019; dp-ucMGP, desphospho-uncarboxylated matrix Gla protein; PIVKA-II, protein induced by vitamin K absence. ***P < .001.

COVID-19 patients with poor outcome (1998 pmol/L; 95% CI, 1737 to 2296) compared with those with good outcome (1157 pmol/L; 95% CI, 1022 to 1312; mean fold change, 1.73; 95% CI, 1.43 to 2.08; $P < .001$; Figure 3A), and significance was maintained after adjustments ($P < .001$).

PIVKA-II

PIVKA-II was measured in the first available sample after admission. Levels were normal in 82.1%, mildly elevated in 13.0%, moderately elevated in 4.1%, and severely elevated in .8% of COVID-19 patients not using VKA (Figure 3B). PIVKA-II distribution was comparable between patients with good (78.6%, 15.7%, 4.3%, and 1.4%, respectively) and poor outcomes (86.8%, 9.4%, 3.8%, and 0%, respectively). PIVKA-II levels were severely elevated in 100% of COVID-19 patients using VKA.

Desmosine

Sufficient plasma for pDES measurements was available for 127 patients and measured in the first available sample after admission. Three dialysis-dependent patients were excluded from the analysis. pDES levels were significantly higher in COVID-19 patients (380 ng/L; 95% CI, 355 to 405) compared with age-dependent reference values of never-smokers (243 ng/L; 95% CI, 228 to 260; mean fold change, 1.56; 95% CI, 1.42 to 1.71; $P < .001$) and former or current smokers (278 ng/L; 95% CI, 260 to 296; mean fold change, 1.37; 95% CI, 1.25 to 1.50; $P < .001$; Figure 4A) [24]. pDES levels, corrected for age, were significantly higher in COVID-19 patients with poor (430 ng/L; 95% CI, 384 to 481) compared with good outcomes (342 ng/L; 95% CI, 310 to 379; mean fold change, 1.25; 95% CI, 1.07 to 1.47; $P = .004$). dp-ucMGP levels significantly correlated with pDES (n = 124; r = .47; $P < .001$; Figure 4B).

CT Assessment

CT scans were available for 109 patients, and CAC and TAC scores were successfully determined for 107 of these patients. TAC and CAC scores were significantly higher in COVID-19 patients with poor outcome compared with those with good outcome; however, both lost significance after adjustments (Supplementary Materials). The association between pulmonary involvement on CT and time-matched dp-ucMGP levels was not significant (n = 109; r = .18; $P = .06$). dp-ucMGP was significantly associated with TAC scores (n = 107; r = .36; $P < .001$) and CAC scores (n = 107; r = .30; $P = .002$).

DISCUSSION

dp-ucMGP, which indirectly indicates extrahepatic vitamin K insufficiency, was severely elevated in hospitalized COVID-19 patients. Impaired MGP activation was associated with poor outcome and accelerated elastic fiber degradation. In contrast, procoagulant FII activity remained preserved.

dp-ucMGP, as a measure of extrahepatic vitamin K status, is a relevant parameter given its close association with mortality [22]. Low dietary vitamin K intake and VKA use are evident causes of elevated dp-ucMGP [15, 22]. Pathological processes leading to upregulation of vitamin K–dependent protein production and resulting in accelerated use of vitamin K

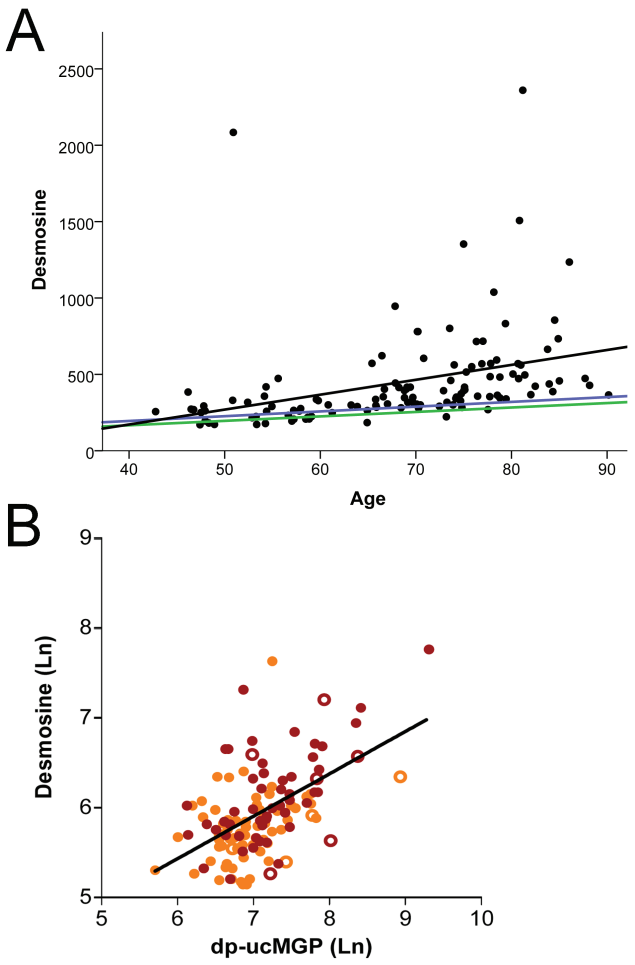

Figure 4. Correlation between dp-ucMGP and desmosine. *A,* Scatterplot showing circulating desmosine levels in those patients aged >40 years (n = 121) by age. The black line represents the deduced equation for coronavirus disease 2019 (COVID-19) patients. The green and blue lines represent Huang et al's [24] calculated equations for nonsmoking and smoking controls, respectively. *B,* For all COVID-19 patients who were not dialysis-dependent at admission with a good outcome (discharge without invasive ventilation, n = 69; orange) or poor outcome (invasive ventilation and/or death, n = 58; red) log-transformed baseline dp-ucMGP and desmosine values are shown, with open circles representing vitamin K antagonist users. The black line represents a linear regression analysis. Abbreviation: dp-ucMGP, desphospho-uncarboxylated matrix Gla protein.

for carboxylation may be another important reason for severe extrahepatic vitamin K insufficiency in COVID-19. Vitamin K supplementation reduced dp-ucMGP in various cohorts [22, 27], with favorable effects on clinically relevant outcome measurements [27]. It is reasonable to assume that vitamin K administration reduces dp-ucMGP in COVID-19. Whether improving dp-ucMGP results in a better outcome of COVID-19 remains to be evaluated.

Intriguingly, many comorbid conditions related to poor COVID-19 clinical outcomes are associated with compromised vitamin K status [1, 7, 18]. The same holds true for ageing [1]. Elastic fiber calcification and degradation are closely related processes [11, 12]. There seems to be an association between vascular mineralization and lung pathologies [28–30]. We demonstrated accelerated elastic fiber degradation and arterial calcification correlating with dp-ucMGP, suggesting interrelationships between vitamin K shortage, insufficient MGP carboxylation, and elastic fiber pathologies in COVID-19.

Although significance was lost after adjustment for age, sex, and VKA use, we found enhanced TAC and CAC in patients with poor prognosis. Hypertension, diabetes, cardiovascular disease, and older age are associated with remodeling of elastic tissues [7]. These damaged and calcified elastic fibers are more prone to further degradation than intact fibers [11]. We speculate that this preexisting elastic fiber dysfunction renders them more susceptible to degradation following enhanced proteolytic activity during COVID-19 [14].

We did not find a significant correlation between dp-ucMGP and pneumonia severity. It is possible that the correlation is confounded by the fact that those with preexisting conditions are predisposed to both higher dp-ucMGP and the development of respiratory failure with less pulmonary involvement. Furthermore, CT severity is a dynamic process that may change rapidly [31]. A clinical trial in which change of both vitamin K status and CT severity are simultaneously assessed before and after vitamin K supplementation would be a more suitable analysis.

Vitamin K1, the main source of vitamin K in the Netherlands, is preferentially transported to the liver, implying that the grade of carboxylation is usually higher for hepatic than extrahepatic vitamin K–dependent proteins (Figure 1) [3, 4, 32]. This likely explains why dp-ucMGP was severely elevated while PIVKA-II was normal in the majority of patients. Similar to MGP, the activation of endothelial protein S is disproportionally impacted in times of vitamin K deficiency. Theoretically, these observations could be compatible with enhanced thrombogenicity in COVID-19 [2], where autopsies revealed bilateral deep venous leg thrombosis in all thromboembolic cases and thrombosis of the prostatic venous plexus in the majority of men who died [33]. Future research should investigate this; however, there is currently no readily available assay to measure carboxylated (active) vs uncarboxylated (inactive) protein S. Enhanced thrombosis in a state of vitamin K deficiency has previously been described in calciphylaxis [5]. Calciphylaxis is characterized by cutaneous blood vessel occlusion due to calcification, leading to ischemic skin infarction [5]. Increased levels of inactive MGP are found in skin tissues and the circulation of calciphylaxis patients [5]. It may be speculated that, analogous to calciphylaxis, impaired local anticoagulant activity due to vitamin K insufficiency is responsible for microvessel thrombosis in COVID-19 [34].

The major strength of our study is the use of robust biomarkers and quantitative CT assessment. Our findings are

limited by the fact that it is impossible to determine which proportion of circulating dp-ucMGP and DES levels originated from the lungs, as both biomarkers are not tissue-specific. Therefore, there is an urgent need for experimental data to better link vitamin K insufficiency specifically with COVID-19–related lung pathologies.

As low vitamin K levels are found in comorbidities that are related to poor outcome of COVID-19 [1, 7], we were unable to formally determine whether vitamin K insufficiency truly predisposes patients to the development of severe COVID-19 or whether it is merely an epiphenomenon. However, the latter seems highly unlikely given the extreme elevation of dp-ucMGP levels in COVID-19 patients, which was much more pronounced than in hypertensive, diabetic, cardiovascular, and COPD patients without COVID-19 (Supplementary Table 1). The strong correlation that we found between vitamin K status and the rate of elastic fiber degradation also suggests causality.

We had to make use of a historical control group due to the implementation of quarantines and social distancing practices to contain SARS-CoV-2. We do not consider this to be a significant problem, however, as dp-ucMGP levels of our historical controls were higher than previously reported in large groups of controls (Supplementary Table 2). Furthermore, differences in dp-ucMGP levels between COVID-19 patients and controls were of such a magnitude that loss of significance when comparing to a matched control group would be highly unlikely.

In conclusion, dp-ucMGP was strongly elevated in hospitalized COVID-19 patients, which indirectly indicates extrahepatic vitamin K insufficiency. Impaired MGP activation was associated with poor outcomes. COVID-19 patients with premorbid elastic fiber pathologies appeared, in particular, to be at increased risk of a complicated disease course. Despite extrahepatic vitamin K deficiency, hepatic prothrombin activation remained preserved. Taken together, these data suggest a mechanism of pneumonia-induced extrahepatic vitamin K depletion leading to accelerated elastic fiber degradation and thrombosis formation. An intervention trial is now needed to assess whether vitamin K administration improves outcomes in patients with COVID-19 by increasing pulmonary MGP and endothelial protein S activation.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. R. J. developed the theory. A. S. M. D., R. J., and L. J. S. designed the study. L. J. S., P. L., and C. M. were responsible for the desphospho-uncarboxylated matrix Gla protein and protein induced by vitamin K absence-II measurements. J. M. W. O. and H. D. were responsible for the (iso)desmosine measurements. P. A. J. performed the computed tomography analyses. H. D., E. G. A. K., C. V., M. P. J. V., and J. W. analyzed the data. I. P. performed the statistical analyses. I. P., J. W., and R. J. wrote the first draft of the manuscript. A. S. M. D., L. J. S., J. M. W. O., P. A. J., T. M. H., R. G., L. E. M. K., and E. F. M. W. critically revised the manuscript.

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Potential conflicts of interest. L. J. S. reports a consultancy fee from Immunodiagnostic Systems and grants from NattoPharma. J. M. W. O. and R. J. are owners of Desmosine.com. R. J. is owner of Emphysema Solutions BV and discloses application of a patent on vitamin K in coronavirus 2019 for prognostic and therapeutic purposes. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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