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Reduced Vitamin K Status as a Potentially Modifiable Prognostic Risk Factor in Covid-19

Anton S.M. Dofferhoff[1*], M.D., Ianthe Piscaer[2*], M.D., Leon J. Schurgers[3*], Ph.D., Jona Walk[1*], M.D., Jody M.W. van den Ouweland[4], Ph.D., Tilman M. Hackeng[3], Ph.D., Pim A. de Jong[5], M.D., Reinoud Gosens[6], Ph.D., Petra Lux[3], Henny van Daal[4], Cecile Maassen[3], Esther G.A. Karssemeijer[1], M.D., Loes E.M. Kistemaker[6], Ph.D., Cees Vermeer[3], Ph.D., Emiel F.M. Wouters[2,7], M.D., Rob Janssen[8], M.D.

[1]Department of Internal Medicine, Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands; [2]Department of Respiratory Medicine, Maastricht University Medical Center+, Maastricht, The Netherlands; [3]Department of Biochemistry, Cardiovascular Research Institute Maastricht, Maastricht University, Maastricht, The Netherlands; [4]Department of Clinical Chemistry, Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands; [5]Department of Radiology, University Medical Center Utrecht, The Netherlands; [6]Department of Molecular Pharmacology, University of Groningen, Groningen, The Netherlands; [7]Ludwig Boltzmann Institute for Lung Health, Vienna, Austria; [8]Department of Pulmonary Medicine, Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands; [*]authors contributed equally to this manuscript

Correspondence: R. Janssen, M.D., Ph.D., Department of Pulmonary Medicine, Canisius-Wilhelmina Hospital, Weg door Jonkerbos 100, 6532 SZ Nijmegen, The Netherland. Telephone: +31-24-3658756. Mail: rob.janssen@cwz.nl

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Abstract

Background A significant proportion of SARS-CoV-2-infected patients develops respiratory failure. Thromboembolism is also prevalent in coronavirus disease 2019 (Covid-19). Vitamin K plays a role in coagulation and possibly also in lung diseases. We therefore hypothesized that vitamin K is implicated in Covid-19 pathogenesis.

Methods 134 Covid-19 patients and 184 controls were included. Inactive vitamin K-dependent matrix Gla protein (*i.e.* dp-ucMGP) and prothrombin (*i.e.* PIVKA-II) were measured, which are inversely related to respectively extrahepatic and hepatic vitamin K status. Desmosine was measured to quantify elastic fiber degradation. Lung involvement and arterial calcifications severity were assessed by computed tomography.

Results Dp-ucMGP was elevated in Covid-19 patients compared to controls (P=0.001). Higher dp-ucMGP was found in Covid-19 patients with poor compared to better outcomes (P=0.002). PIVKA-II was normal in 81.8%, mildly elevated in 14.0% and moderately elevated in 4.1% of Covid-19 patients not using vitamin K antagonists. Dp-ucMGP in Covid-19 patients was correlated with desmosine (P<0.001), thoracic aortic calcification (P<0.001) but not with pneumonia severity.

Conclusions Extrahepatic vitamin K status was severely reduced in Covid-19 patients, as reflected by elevated inactive MGP, and related to poor outcome. Procoagulant prothrombin activity remained preserved in the majority of Covid-19 patients, which is compatible with the increased thrombogenicity that is frequently observed in severe Covid-19. Impaired MGP activation was linked to accelerated elastic fiber degradation and premorbid vascular calcifications. A trial should assess whether increasing MGP and protein S activity by vitamin K administration improves Covid-19 outcomes.

Introduction

Coronavirus disease 2019 (Covid-19) is an infectious disease caused by severe acute respiratory syndrome (SARS) coronavirus (CoV)-2.[1] The majority of individuals who contract SARS-CoV-2 have mild symptoms.[2] However, a significant proportion develops respiratory failure due to pneumonia and/or acute respiratory distress syndrome (ARDS).[3] Covid-19 may also have extrapulmonary manifestations. Coagulopathy and venous thromboembolism are prevalent in severe SARS-CoV-2 infections and are associated with decreased survival.[4,5] The mechanisms that activate coagulation in Covid-19 are not known at present but appear to be linked to inflammatory responses rather than specific properties of the virus.

Coagulation is an intricate balance between clot promoting and dissolving processes in which vitamin K plays a well-known role. Coagulation factors II (FII; *i.e.* prothrombin), VII, IX and X depend on vitamin K for carboxylation to fulfill their primary biological function. Vitamin K is also cofactor of anticoagulant proteins C and S. In contrast to vitamin K-dependent procoagulant factors and protein C, a significant proportion of protein S is extrahepatically synthesized in endothelial cells, which plays a local suppressive role against thrombosis formation in blood vessels.[6] Carboxylation during vitamin K deficiency is more severely compromised for extrahepatic than hepatic vitamin K-dependent proteins (Fig. 1).[7] This can paradoxically lead to enhanced thrombogenicity in a state of low vitamin K.[8]

Matrix Gla protein (MGP) is also vitamin K-dependent but not involved in coagulation.[9] MGP is well-known as a calcification inhibitor in arterial walls.[10] However, it is also strongly expressed in lungs.[11] MGP's role in the pulmonary compartment seems to be comparable with that in the vasculature.[12] Elastic fibers are essential components of the extracellular matrix in lungs and have high affinity for calcium.[13,14] MGP is crucial for the protection of elastic fibers

against calcification.[9] Degradation, fibrosis and mineralization of elastic fibers are interrelated remodeling processes, as synthesis of matrix metalloproteinases (MMPs) and release of latent transforming growth factor (TGF)-β from the extracellular matrix are enhanced in parallel with elastic fiber calcification.[15-17] These processes also involve partially degraded elastic fibers becoming prone to mineralization due to increased polarity.[14] Preliminary data show that a subset of pulmonary macrophages, which produce MMPs and play a role in lung fibrosis,[18] are increased in severe SARS-CoV-2 pneumonia.[19] Covid-19 may theoretically be linked to both vitamin K deficiency and elastic fiber metabolism through a series of sequential pathologic steps (Fig. 2).

Vitamin K deficiency may be presumed to worsen Covid-19 outcome. Individuals with severe SARS-CoV-2 infections often have comorbidities that are also associated with reduced vitamin K status, such as hypertension, diabetes and cardiovascular diseases.[10,20] The body uses vitamin K very efficiently, and storage capacity is low.[21] There are reasons to suspect enhanced utilization of vitamin K for carboxylation of pulmonary MGP and coagulant factors in Covid-19.[4,8,22-24] Depletion of vitamin K may have devastating consequences in lungs,[25] and it has been suggested that these effects may be very acute.[26]

The aim of this study was to evaluate whether a reduced vitamin K status plays a role in the pathogenesis of Covid-19 thereby linking, in particular, pulmonary and coagulopathic disease manifestations.

Methods

Subjects

134 subjects hospitalized for Covid-19 in the Canisius-Wilhelmina Hospital in Nijmegen, The Netherlands, between March 12th and April 11th 2020 were included for analysis. SARS-CoV-

2 infection was confirmed by Real Time polymerase chain-reaction (RT-PCR) testing in all study subjects. Data on patient comorbidities were extracted from hospital admission records, and vitamin K antagonist (VKA) usage was determined based on records from pharmacies and anticoagulant clinics. The study was approved by the United Medical Research Ethics Committees of the Canisius-Wilhelmina Hospital (CWZ-nr. 027-2020; date of approval 12th March 2020). The need for written informed consent was waived by the committee. There was, however, an opt-out possibility for patients after they were informed about the study.

A total of 184 age-matched control subjects from a previous COPD study were included in addition (www.controlled-trials.com, identifier ISRCTN86049077).[27] Covid-19 and control subjects where use of VKA was unknown were excluded from the analysis.

Patients were followed-up until they reached one of three endpoints: 1) discharge from the hospital, 2) admission to the intensive care unit (ICU) for intubation and mechanical ventilation, or 3) death. 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 either required intubation and mechanical ventilation or died due to Covid-19.

Quantification of dp-ucMGP

Although technically feasible, direct quantification of blood vitamin K levels would not have been an appropriate method to assess overall vitamin K status in our study due to differences in bioavailability and half-life time between the two naturally occurring vitamin K forms (*i.e.* vitamin K1 and K2). Additionally, the intake of vitamin K2, a group name of all menaquinones, is too low to measure accurately. Measuring inactive levels of vitamin K-dependent protein in the circulation is a valuable method for quantifying the combined deficit of vitamin K1 and K2. Desphospho-uncarboxylated (dp-uc)MGP (*i.e.* inactive MGP) may be considered as the most appropriate surrogate marker of extrahepatic vitamin K status in

Covid-19.[23,24] Subjects 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 CE marked chemiluminescent InaKif MGP assay on the IDS-iSYS system (IDS, Boldon, UK).[28] In brief, 50 µL of patient sample or calibrators were incubated with magnetic particles coated with murine monoclonal dpMGP antibody, an acridinium labelled murine monoclonal ucMGP antibody and assay buffer. The magnetic particles were captured using a magnet and a wash step performed to remove any unbound analyte. Trigger reagents were added. The resulting light emitted by the acridinium label is directly proportional to the concentration of dp-ucMGP in the sample. The within-run and total precision of this assay were 0.8 – 6.2% and 3.0 – 8.2%, respectively. The assay measuring range is between 200 – 12,000 pmol/L and was found to be linear up to 11,651 pmol/L.

Maximum dp-ucMGP's were used for comparisons between groups, and baseline values were used for correlations of dp-ucMGP with blood and radiological biomarkers. Dp-ucMGP values below 300 pmol/L are considered to be in the normal healthy range.

PIVKA-II

Protein induced by vitamin K absence (PIVKA)-II (*i.e.* ucFII) was used to assess hepatic/procoagulant vitamin K status. Subjects with high PIVKA-II levels have low hepatic vitamin K status and *vice versa*.

Circulating PIVKA-II levels were measured using a conformation-specific monoclonal antibody in an ELISA-based.[29] Results are expressed as arbitrary units per liter (AU/mL) as in states of vitamin K deficiency circulating ucFII may comprise multiple forms of partially carboxylated FII and neither their relative abundance in serum nor their relative affinity for

the antibody is known. Using electrophoretic techniques 1 AU is equivalent to 1 mg of purified ucFII. The detection limit, as well as upper limit of normal, was 0.15 AU/mL ucFII in serum;[29] 0.15-0.5 AU/mL is mildly, 0.5-2.0 moderately and >2.0 is severely elevated.

Desmosine

Plasma (p) desmosine and isodesmosine (DES) levels were used as a marker for the rate of elastic fiber degradation.[30] DES are formed during the cross-linking of tropo-elastin polymers and are released in the bloodstream after degradation of elastic fibers.[30,31] pDES is therefore positively associated with the rate of systemic elastic fiber degradation.

DES fractions were measured using liquid chromatography-tandem mass spectrometry with deuterium-labelled desmosine as internal standard, as previously described.[27,30] Coefficient of variations of intra- and inter-assay imprecision were <8.2%, lower limit of quantification of 140 ng/L, and assay linearity up to 210,000 ng/L.

For each pDES measurement in a Covid-19 patient, virtual age-matched pDES values were calculated using published pDES equations: (50+2.91*age for never-smokers and 70+3.12*age for ever smokers).[30]

CT acquisition

Thin slice CT scans were acquired by using a Philips Ingenuity multi-detector row scanner (Philips Healthcare). CT images of 1-mm thickness were reconstructed by using iterative model-based reconstruction in the axial plane. A low-dose scanner protocol was used with 100 kVp and variable mAs without intravenous contrast administration.

CT lung assessment

Quantitative measurements of the volume of ground glass and consolidation were undertaken using the Intellispace Portal (COPD package, Instellispace version 10, Philips Healthcare). In the software, first the lungs were segmented from the chest wall and major vessels and bronchi. Manual adjustments were implemented by a board-certified chest radiologist where required, given the extensive lung consolidation. Subsequently, the lung voxels were counted to derive a total lung volume in milliliters. Diseased lungs were defined as those voxels with an attenuation of Hounsfield Units (HU) > -700 as previously defined for interstitial lung disease.[32] Visually this corresponded favorably to the COVID related abnormalities. The abnormal voxels were expressed as a percentage of the total volume as a percentage diseased lung. Additionally, a percentile method was employed, where the HU value at the 85th percentile was used.[33] Given that air has a HU of -1000 and water a HU of 0, the more the lung is diseased, the higher the HU value.

CT vascular assessment

Coronary and aortic calcifications were quantified in the Intellispace Portal (Heartbeat CS package, Instellispace version 10, Philips Healthcare). Calcifications were defined as dense areas with a HU of 130 and higher. The calcifications were visually localized up to the arterial wall by a board-certified chest radiologist, who semi-automatically segmented the calcifications. The volume of calcifications was used as a measure of calcification burden.

Statistical analysis

Statistical analyses were performed using SPSS (version 24, IBM, Chicago, IL, USA). Analysis of variance (ANOVA) was used to compare dp-ucMGP levels between Covid-19 patients and controls as well as to compare dp-ucMGP and radiological scores between Covid-19 patients with good and poor outcomes, respectively. In subjects with Covid-19, the correlation between dp-ucMGP and pDES was assessed using Pearson's correlation

coefficient. Pearson's correlation coefficient was also used for the association between dp-ucMGP and Covid-19 severity score, coronary artery calcium (CAC) score and thoracic aortic calcium (TAC) score on CT. Full factorial (including all interactions for fixed factors) analysis of covariance (ANCOVA) was used to perform aforementioned dp-ucMGP and radiological analyses adjusted for age, gender and use of VKA. pDES was adjusted for age in the comparison between Covid-19 patients and reference values as well as between Covid-19 patients with good and poor outcomes.

Dp-ucMGP, pDES and radiological scores had a log-normal distribution and were therefore natural log-transformed prior to analyses. The mean difference and 95% CI of the log-transformed values was back-transformed to the mean fold change.

Use of VKA is associated with extremely high PIVKA-II and, therefore, users of VKA were separately assessed in the analysis with PIVKA-II as variable. Dialysis has a strong influence on pDES, and therefore, patients receiving dialysis at baseline were excluded from analysis involving pDES.

Normally distributed continuous variables are presented as mean ± standard deviation (SD), whereas continuous variables with a natural-log distribution were presented as back-transformed mean and 95% CI. A P-value of <0.05 was used as threshold for statistical significance.

Results

The mean age of COVID-19 patients was 68±12 years, 93 (70%) were male and 12 (9.0%) used VKA. Of the historical controls, 85 (46%) were male, 3 subjects (1.6%) were currently taking VKA, and mean age was 61±6.5 years. Characteristics are shown in Table 1.

Dp-ucMGP

Dp-ucMGP levels were significantly higher in Covid-19 patients (1482 pmol/L, 95% CI, 1346 to 1633 pmol/L) compared to healthy controls (471 pmol/L, 95% CI, 434 to 511 pmol/L, mean fold change 3.15, 95% CI, 2.78 to 3.58, P<0.001, Fig. 3A), which remained significant after adjustments (P=0.001). Dp-ucMGP levels were significantly higher in Covid-19 patients with poor outcome (1998 pmol/L, 95% CI, 1737 to 2298 pmol/L) compared to those with good outcome (1163 pmol/L, 95% CI, 1027 to 1319, mean fold change 1.72, 95% CI, 1.42 to 2.07, P<0.001; Fig. 3A), and significance was maintained after adjustments (P=0.002).

PIVKA-II

PIVKA-II levels were normal in 81.8%, mildly elevated in 14.0% and moderately elevated in 4.1% of Covid-19 patients not using VKA (Fig. 3B). In Covid-19 patients with good outcome and not using VKA, PIVKA-II levels were normal in 79.4%, mildly elevated in 16.2% and moderately elevated in 4.4%. In Covid-19 patients with poor outcomes and not using VKA, PIVKA-II levels were normal in 84.9%, mildly elevated in 11.3% and moderately elevated in 3.8%.

PIVKA-II levels were severely elevated in 100% of Covid-19 patients using VKA.

Desmosine

pDES levels were significantly higher in Covid-19 patients (0.38 ng/L, 95% CI, 0.35 to 0.40 ng/L) compared to age-dependent reference values of never-smokers (0.24 ng/L, 95% CI, 0.23 to 0.26 ng/L; mean fold change 1.55, 95% CI, 1.41 to 1.71, P<0.001) and former or current smokers (0.28 ng/L, 95% CI, 0.26 to 0.30 ng/L, mean fold change 1.36, 95% CI 1.24 to 1.50, P<0.001; Fig. 4A).[30] pDES levels, corrected for age, were significantly higher in Covid-19 patients with poor (0.43 ng/L, 95% CI 0.38 to 0.48 ng/L) compared to good

outcomes (0.34 ng/L, 95% CI 0.31 to 0.38 ng/L; mean fold change 1.26, 95% CI, 1.08 to 1.48, P=0.004).

Dp-ucMGP was significantly associated with pDES (n=123, r=0.51, P<0.001; Fig. 4B).

CT assessment

Percentage pneumonia involvement was significantly higher in Covid-19 patients with poor (29.1%, 95% CI, 24.9 to 34.2%) vs. good outcome (21.0%, 95% CI, 18.2 to 24.2%, mean fold change 1.39, 95% CI, 1.12 to 1.72, P=0.003). TAC score was significantly higher in Covid-19 patients with poor (2053, 95% CI, 1120 to 3763) vs. good outcome (754, 95% CI, 402 to 1415, mean fold change 2.72, 95% CI, 1.14 to 6.51, P=0.025), which remained significant after adjustments (P=0.019). CAC score was not significantly different between Covid-19 patients with poor (449, 95% CI, 230 to 878) and good outcomes (235, 95% CI, 115 to 481, mean fold change 1.92, 95% CI, 0.72 to 5.11, P=0.19, P=0.092 after adjustments).

The association between pulmonary involvement on CT and dp-ucMGP levels was not significant (n=108; r= 0.023; P= 0.81). Dp-ucMGP levels were significantly associated with TAC scores (n=106; r= 0.39; P<0.001) but not with CAC scores (n=106; r=0.093; P=0.35).

Discussion

The results of this study demonstrated severely reduced extrahepatic vitamin K status in hospitalized Covid-19 patients. Impaired MGP activation was found to be associated with poor outcome and accelerated elastic fiber degradation. Procoagulant FII activity remained preserved in the majority of Covid-19 patients, which is compatible with the increased thrombogenicity that is frequently observed in severe Covid-19.

Low dietary vitamin K intake and VKA use are evident causes of vitamin K shortage.[21,34] However, ongoing pathological processes leading to upregulation of vitamin K-dependent protein production and causing accelerated utilization of vitamin K for carboxylation may be another important reason for severe vitamin K extrahepatic insufficiency in Covid-19.

Intriguingly, many comorbid conditions, which we and others found to be related to worse Covid-19 clinical outcomes, are associated with compromised vitamin K status.[10,20,27] The same holds true for ageing.[20,35] Vitamin K insufficiency is irrefutably linked to vascular calcifications by reducing active MGP levels required for inhibition of elastic fiber mineralization.[9,10] Circumstantial evidence suggests that similar processes also occur in lungs.[11,12,23-26] There seems to be an association between vascular mineralization and lung pathologies, as both lung fibrosis and emphysema are associated with arterial calcification scores.[36,37] Calcification and degradation of elastic fibers are closely related pathological processes.[15] This is illustrated by the strong correlation between circulating DES levels, which reflect the rate of systemic elastic fiber degradation, and arterial calcification score in COPD.[38] Furthermore, both elastic fiber degradation and arterial calcification are related to all-cause mortality in COPD patients underscoring the clinical relevance of these biomarkers.[38,39] We demonstrated accelerated elastic fiber degradation in Covid-19 and a correlation of circulating dp-ucMGP with DES levels, suggesting an interrelationship between vitamin K shortage, insufficient MGP carboxylation and elastic fiber degradation in Covid-19 patients. We also found enhanced TAC scores on CT in Covid-19 patients with poor prognosis, reflecting preexisting elastic fiber dysfunction. Vitamin K insufficiency could therefore represent a unifying risk factor for Covid-19 disease severity. Hypertension, diabetes, cardiovascular disease and older age are associated with remodeling of elastic tissues.[10] These damaged and calcified elastic fibers are more prone to further degradation than intact fibers.[15,40] We speculate that this pre-existing elastic fiber dysfunction renders

them more susceptible to degradation following enhanced MMP production by macrophages during Covid-19.[18,19]

We did not find a significant correlation between vitamin K status and pneumonia severity on CT. There are various possible explanations for this lack of association. Vitamin K insufficiency in Covid-19 patients is most likely the result of premorbid status and acute modifications secondary to the infection. It is plausible that SARS-CoV-2 infected patients with comorbid conditions develop respiratory failure with less lung involvement than those who are otherwise healthy. Furthermore, CT severity is a dynamic process that may change on a day-to-day basis.[41] 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 method to determine the effect of vitamin K on SARS-CoV-2 pneumonia.

Dp-ucMGP is associated to mortality in various cohorts.[42] Vitamin K supplementation has a reducing effect on dp-ucMGP levels;[34,43,44] the opposite holds true regarding VKA use.[34] Administration of vitamin K has previously demonstrated favorable effects on clinically relevant outcome measures.[43,44] We found very high levels of dp-ucMGP in Covid-19 patients with poor prognosis. It may be expected that vitamin K administration has an improving effect on vitamin K status in Covid-19 patients, this, however, has never been studied. Additionally, it remains to be evaluated whether improving vitamin K status would result in a better prognosis in Covid-19 patients.

Vitamin K1, the main source of vitamin K in The Netherlands,[45] is preferentially transported to the liver, implying that the grade of carboxylation is usually higher for hepatic than extrahepatic vitamin K-dependent proteins (Fig. 1).[6,7,46] This may be the reason that dp-ucMGP was severely elevated, while PIVKA-II was normal in the majority of Covid-19 patients. Furthermore, we assume that vitamin K insufficiency in Covid-19 patients has

greater effects on protein S than on FII production (Fig. 1). This would be compatible with enhanced thrombogenicity in Covid-19.[5] Preferred vitamin K-dependent activation of hepatic procoagulation factors over endothelial protein S would be compatible with findings from an autopsy series revealing bilateral deep venous leg thrombosis in all thromboembolic cases, as well as with thrombosis of the prostatic venous plexus in the majority of men who died of Covid-19.[47] Although increased thrombosis risk in a state of vitamin K insufficiency may sound paradoxical, this phenomenon has previously been described in calciphylaxis, a rare and life-threatening disorder.[8] Calciphylaxis is characterized by the occlusion of cutaneous blood vessels due to calcification, leading to ischemic infarction of the skin.[8] It is noteworthy that increased levels of inactive MGP are found in skin tissues and increased circulating levels of dp-ucMGP are noticed in calciphylaxis patients.[8,48] Anticoagulant activity is impaired in calciphylaxis, similar to what we found in Covid-19, with thrombosis of microvessels as key histopathological features of both calciphylaxis and Covid-19 in skin and lungs, respectively.[8,49]

VKA form a class of anticoagulant drugs that reduce the activity of procoagulation factors, as well as of other vitamin K-dependent proteins, by interfering with vitamin K metabolism. In line with our findings of compromised anticoagulant and relatively spared procoagulant activity during vitamin K insufficiency in Covid-19, stroke risk paradoxically increases in the first days following VKA initiation in atrial fibrillation patients.[50] Calciphylaxis risk and mortality is also significantly increased by VKA use,[8] and VKA is related to reduced survival in idiopathic pulmonary fibrosis.[25] Anticoagulants have also been shown to be associated with the risk of Covid-19 in a recent study.[51] This, however, did not specify use of VKA or non-VKA anticoagulants.[51] A proof-of-concept study on vitamin K1 supplementation in calciphylaxis is currently ongoing.[8] We propose that such a trial should also be conducted in Covid-19.

The major strengths of our study are the thorough characterization of the Covid-19 patients included, use of robust biomarkers to quantify hepatic and extrahepatic vitamin K status, automated assessment of CT scans, and presentation of data suggesting relevant underlying disease mechanisms. However, there were also some limitations that should be addressed. It was impossible to determine which proportion of circulating dp-ucMGP and DES levels originated from the lungs, as both biomarkers are not tissue specific. There is urgent need for experimental data to better link vitamin K insufficiency specifically with Covid-19-related lung pathologies. Furthermore, we did not have the availability of a test to quantify protein S levels that have not been activated by vitamin K. Given the extreme extrahepatic vitamin K insufficiency in Covid-19, however, it seems reasonable to assume that carboxylated protein S levels in Covid-19 patients are reduced. As low vitamin K levels are found in comorbidities that are related to poor outcome of Covid-19,[10,20] another limitation is that we were unable formally to 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 and cardiovascular patients without Covid-19 (Supplementary Table 1). The strong correlation, which 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 the Covid-19 pandemic. We do not consider this to be a major problem, however, as dp-ucMGP levels of our historical controls were poorer 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, extrahepatic vitamin K status was severely compromised in Covid-19 and lower in patients with a poor outcome compared to those with good outcome. Covid-19 patients with premorbid elastic fiber pathologies appeared, in particular, to be at increased risk of complicated disease course. Extrahepatic/procoagulant prothrombin activation remained preserved. The data provided suggest potential mechanistic links between reduced vitamin K status, lung tissue injury and thrombogenicity in Covid-19. An intervention trial is now needed to assess whether vitamin K administration improves outcome in patients with Covid-19.

Authors' contributions

RJ developed the theory. ASMD, RJ and LJS designed the study. LJS, PL and CM were responsible for the dp-ucMGP and PIVKA-II, and JMWO and HD for the DES measurements. PAJ performed the CT analyses. JW, HD, EGAK and CV analyzed the data, and IP performed the statistical analysis. RJ, IP, and JW wrote the first draft of the manuscript. ASMD, LJS, JMWO, TMH, RG, LEMK, and EFMW critically revised the manuscript.

Conflict of interest statements

LJS reports consultancy fee from Immunodiagnostic systems and grants from NattoPharma. JMWO and RJ are owners of Desmosine.com and RJ is owner of Emphysema Solutions BV. RJ discloses application of a patent on vitamin K in Covid-19 for prognostic and therapeutic purposes. ASMD, IP, JW, TMH, PAJ, RG, PL, HD, CM, EGAK, LEMK, CV, and EFMW declare no competing interests.

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None

Ethics committee approval

The local review committee of the Canisius-Wilhelmina Hospital approved the protocol (CWZ-nr. 027-2020).

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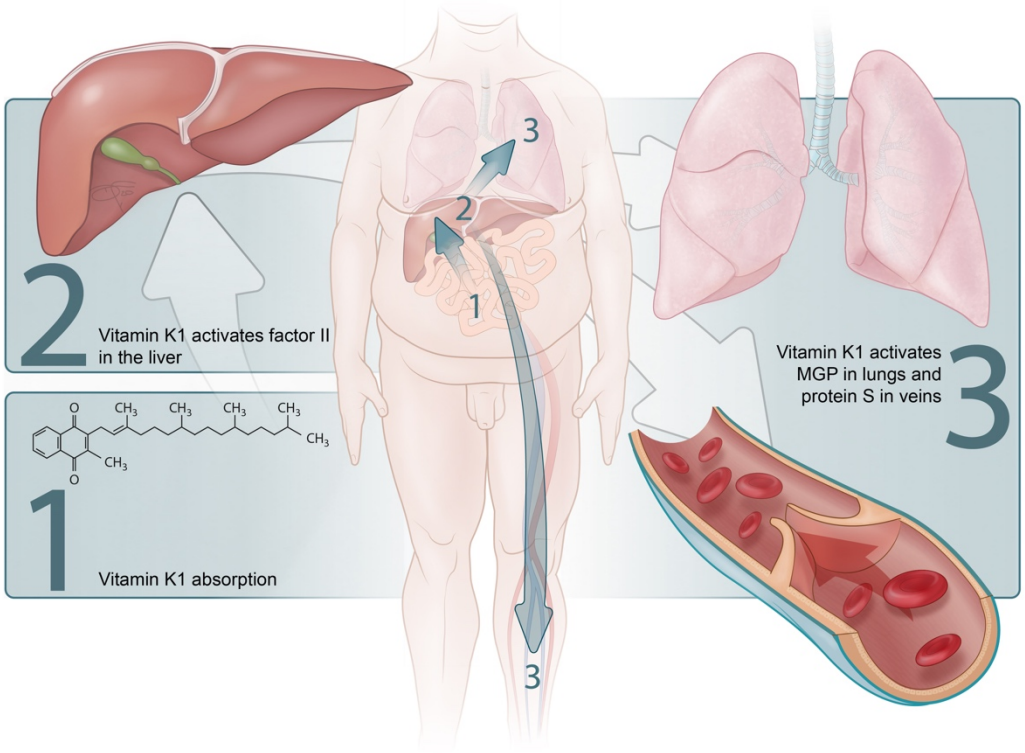
Table 1: Baseline characteristics of Covid-19 patient and healthy control cohorts.

	Covid-19		Controls
	Good outcome	Poor outcome	
Subjects	74	60	184
Age (years)	64±13	72±10	61±6.5
Male (%)	46 (62)	47 (78)	85 (46)
VKA use (%)	5 (6.8)	7 (12)	3 (1.6)
Hypertension (%)	28 (38)	22 (35)	-
Self-reported hypertension (%)	-	-	41 (22)
Measured hypertension (%)*	-	-	96 (52)
Diabetes mellitus (%)	15 (20)	15 (25)	14 (7.6)
Cardiac or cardiovascular disease (%)	17 (23)	21 (35)	12 (6.5)
Asthma/COPD (%)	13 (18)	12 (20)	7 (3.8)
Other respiratory disease (%)	5 (6.8)	10 (17)	3 (1.6)
Immunocompromised (%)	4 (5.4)	2 (3.3)	0 (0)
Dialysis dependent (%)**	1 (1.4)	2 (3.3)	0 (0)
Active malignancy (%)	6 (8.1)	6 (10)	0 (0)

Covid-19: Coronavirus 2019; *VKA*: Vitamin K antagonist; *COPD*: chronic obstructive pulmonary disease**;** * Systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg; ** At admission

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 utilized 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 matrix Gla protein (MGP).

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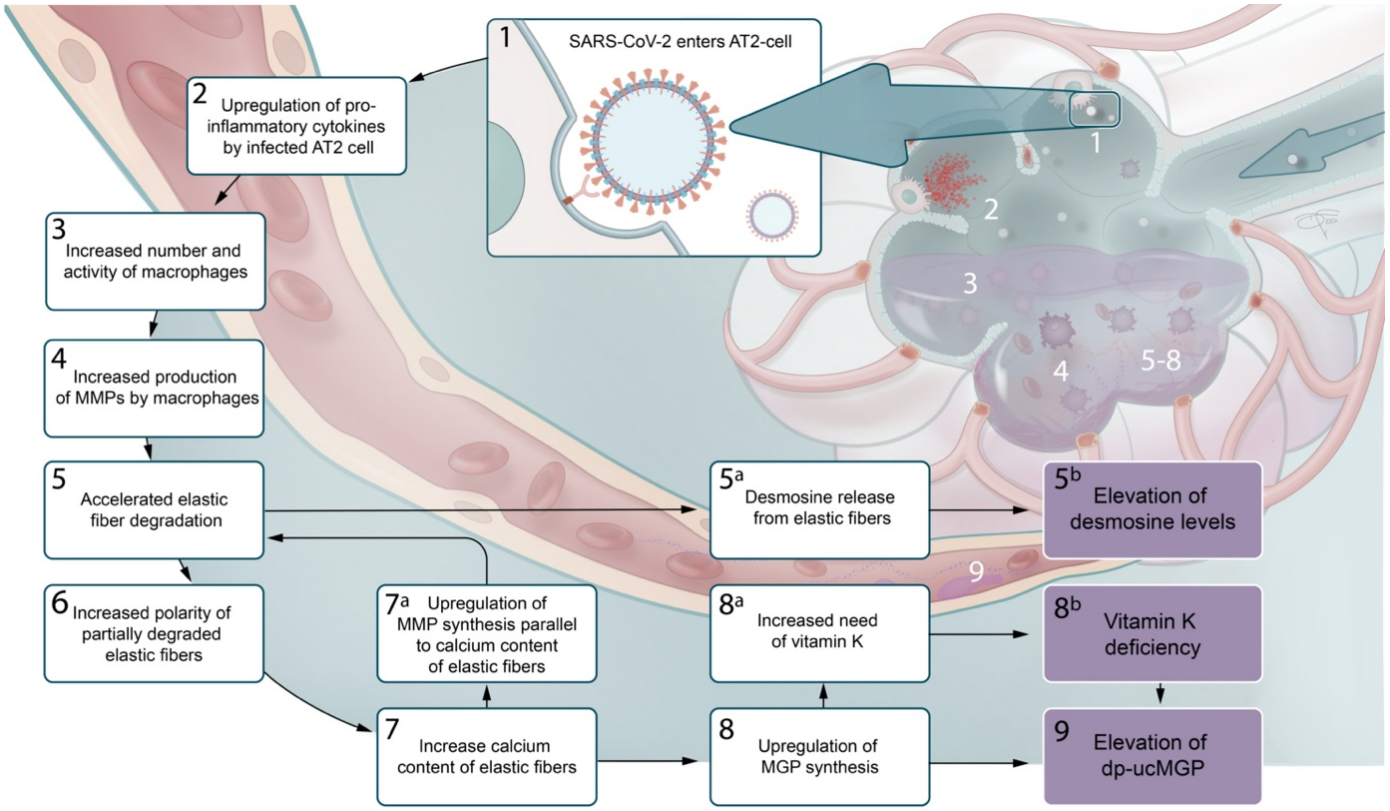
Peer-reviewed version available at Clinical Infectious Diseases 2020; doi:10.1093/cid/ciaa1258

Figure 2. Proposed sequential pathologic steps linking SARS-CoV-2 pneumonia to vitamin K insufficiency and accelerated elastic fiber degradation

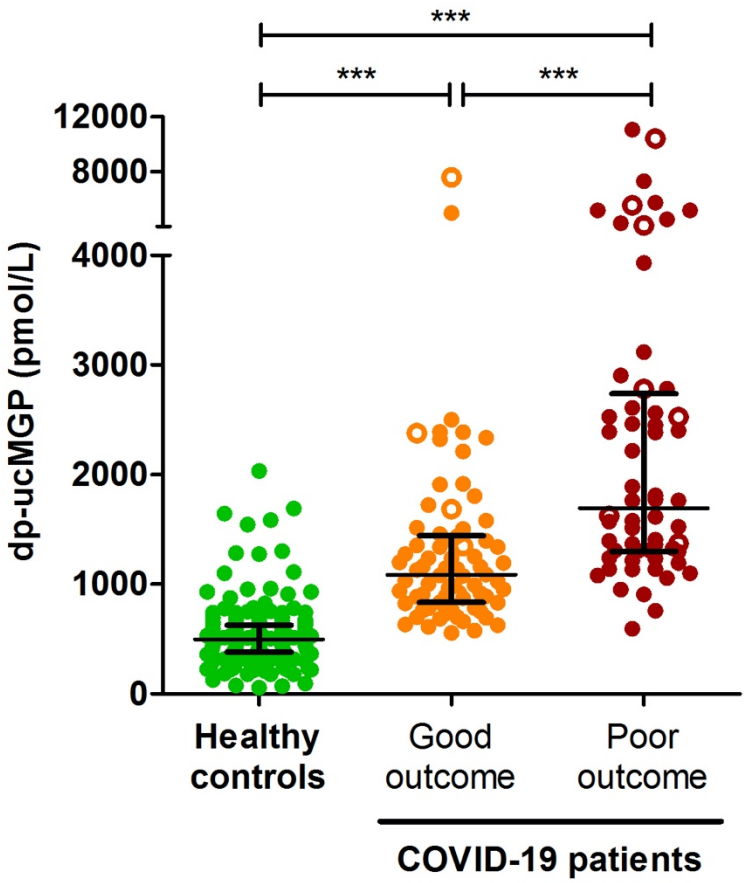


(1) Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) enters alveolar type II (AT2) cell. (2) The infected AT2 cell responses by upregulating synthesis of proinflammatory cytokines. (3) This leads to an increase in the number and activation of pulmonary macrophages. (4) These infiltrating macrophages produce matrix metalloproteinases (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 calcium content, which further accelerates elastic fiber degradation in a self-propagating vicious circle. (8) Matrix Gla protein (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 utilization 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 desphospho-uncarboxylated (dp-uc)MGP in lungs and blood.

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 mechanical ventilation, n=74, orange) or poor outcome (mechanical ventilation and/or death, n=60, red), compared to a cohort of healthy controls. Subjects with high dp-ucMGP have low extrahepatic vitamin K status and *vice* versa. The maximal dp-ucMGP measured during the study is shown, with open circles representing those patients using VKA at admission. **(B)** PIVKA-II was measured in plasma at baseline in those patients not using VKA (n=121). The normal range for healthy controls is shown in gray.

A.



B.

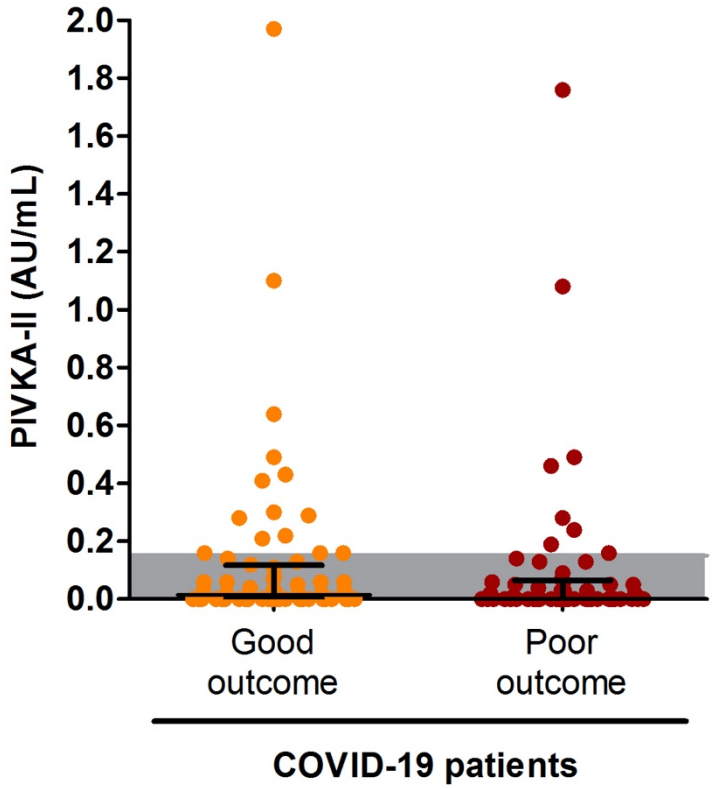
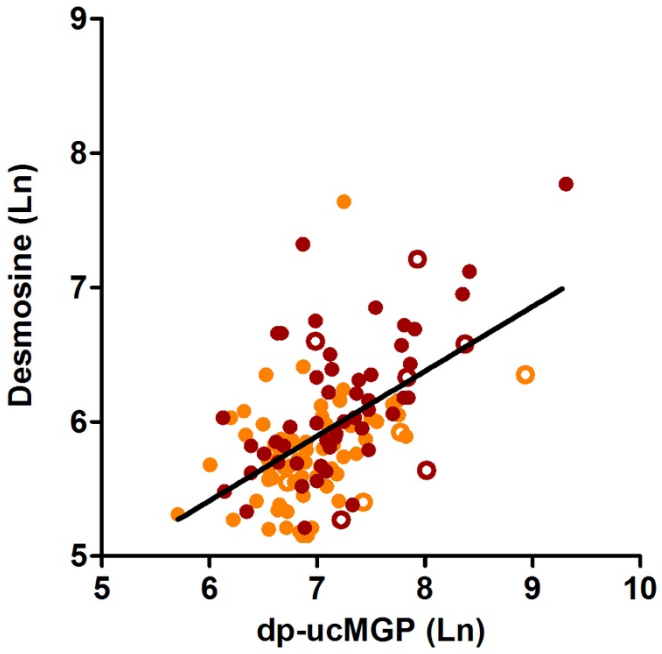


Figure 4: Correlation between dp-ucMGP and desmosine. (A) For all Covid-19 patients who were not dialysis dependent at admission with a good outcome (discharge without mechanical ventilation, n=73, orange) or poor outcome (mechanical ventilation and/or death, n=58, red) log-transformed baseline dp-ucMGP and desmosine values are shown, with open circles representing VKA users. The black line represents a linear regression analysis. **(B)** Scatterplot showing circulating desmosine levels in those patients over 40 years old (n=128) by age, the black line represents the deduced equation for Covid-19 patients. The green and blue lines represent Huang *et al*'s calculated equations for non-smoking and smoking controls, respectively.

A.



B.