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

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Abstract

Introduction: Coronavirus 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus-2. The majority of patients have at most mild symptoms, however, a significant proportion develops respiratory failure. COVID-19 may also progress beyond the lungs. Coagulopathy and thromboembolism are prevalent in severe COVID-19 and relate to decreased survival. Coagulation is an intricate balance between clot promoting and dissolving processes in which vitamin K plays a well-known role. We hypothesized that vitamin K status is reduced in patients with severe COVID-19. **Methods:** Vitamin K status was assessed by measuring desphospho-uncarboxylated matrix Gla protein (dp-ucMGP; inversely related to vitamin K status) and the rate of elastin degradation by measuring desmosine. We included 123 patients who were admitted with COVID-19 and 184 controls. **Results:** Dp-ucMGP levels were significantly elevated in COVID-19 patients (1,673±1,584 pmol/L) compared to controls (536±291 pmol/L; $p<0.0005$). Dp-ucMGP levels were significantly higher in COVID-19 patients with unfavorable outcome compared to those with less severe disease. Furthermore, dp-ucMGP and desmosine levels were significantly associated ($r=0.65$; $p<0.0005$). **Conclusions:** Vitamin K status was reduced in patients with COVID-19 and related to poor prognosis. Also, low vitamin K status seems to be associated with accelerated elastin degradation. An intervention trial is now needed to assess whether vitamin K administration improves outcome in patients with COVID-19.

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Introduction

Coronavirus 2019 (COVID-19) is an infectious disease caused by the severe acute respiratory syndrome (SARS) coronavirus (CoV)-2. The majority of individuals who contract SARS-CoV-2 have at most mild symptoms [1], however, a significant proportion develops respiratory failure due to severe pneumonitis and/or acute respiratory distress syndrome (ARDS) [2]. COVID-19 may also have extrapulmonary manifestations. Coagulopathy and venous thromboembolism are prevalent in severe COVID-19 and relate to decreased survival [3,4]. Pathomechanistic links between pulmonary and coagulopathic manifestations of COVID-19 have not yet been entirely elucidated.

Coagulation is an intricate balance between clot promoting and dissolving processes in which vitamin K plays a well-known role. Coagulation factors II (*i.e.* thrombin), VII, IX and X depend on vitamin K for their carboxylation and to fulfill their biological function. Vitamin K antagonists (VKAs) form a class of anticoagulant drugs that reduce activity of these proteins by interfering with vitamin K metabolism. Data about the effects of VKAs in patients with COVID-19 are lacking. However, we may learn from experiences gained in related diseases.

Idiopathic pulmonary fibrosis (IPF) is characterized by progressive loss of lung function. Since the coagulation cascade seems to be involved in its pathogenesis [5], a potential role of anticoagulants in decelerating IPF progression has been studied in two prospective clinical trials [6,7]. The first study had an open-label design and included patients who were admitted with progressive deterioration of IPF [6]. Participants received prednisolone with or without anticoagulation therapy, consisting of low-molecular-weight heparin (LMWH) during hospitalization and VKAs in the outpatient setting [6]. This anticoagulant regime had a reducing effect on mortality related to IPF exacerbations (*i.e.* 18% vs. 71%), which are devastating episodes of sudden disease acceleration [8]. The double-blinded, randomized,

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placebo-controlled ACE-IPF trial was subsequently conducted to further evaluate the role of anticoagulants in IPF [7]. However, this trial was prematurely terminated due to increased risk of mortality in the VKA-treated group (*i.e.* 19% vs. 4%). In contrast to the first study with a favorable effect of anticoagulants [6], participants in the active arm of the ACE-IPF trial did not receive LMWH during hospital admissions but were treated with VKAs for the entire study duration [7]. Adverse effects of VKAs have also been put forward by retrospective studies evaluating survival in IPF patients with a regular indication for anticoagulant therapy [9,10].

Although data about the effects of VKAs in patients with COVID-19 are lacking, we are concerned about their use based on the pathogenic resemblances between IPF and the pulmonary manifestations of COVID-19. LMWH has been studied in COVID-19 and was associated with lower 28-day mortality in SARS-CoV-2-infected patients with signs of coagulopathy [11].

Deficiency of vitamin K might be suspected to be associated with worse COVID-19 outcome, given that patients with severe COVID-19 are more likely to have comorbidities such as diabetes type 2, hypertension and cardiovascular diseases, which are associated with reduced vitamin K status [12-14]. Based on pleiotropic effects of various vitamin K-dependent anticoagulant factors (*i.e.* protein C and S) as well as a protein outside the coagulation cascade (*i.e.* matrix Gla protein (MGP)), it may be suspected that vitamin K might potentially play a modulatory role in the pathogenesis of COVID-19.

Therefore, we hypothesized that vitamin K status could be reduced and inversely related to survival in patients with severe COVID-19.

Methods

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Subjects

123 subjects admitted to the Canisius-Wilhelmina Hospital in Nijmegen, The Netherlands, for COVID-19 between March 12th and April 11th 2020 were included for analysis. SARS-CoV-2 infection was confirmed by polymerase chain-reaction (PCR) testing. Data on patient comorbidities was extracted from hospital admission records, and VKA usage was determined based on records from pharmacies and the anticoagulant clinic (region Nijmegen). The mean age of patients was 68±12 years, 87 (71%) were male and 11 (8.9%) used VKAs. Patient characteristics are shown in *table 1*. The study (CWZ-nr. 027-2020; date of approval 12th March 2020) was approved by the Medical research Ethics Committees United of the Canisius-Wilhelmina Hospital.

In addition, a total of 184 control subjects from a previous study were included (www.controlled-trials.com, identifier ISRCTN86049077) [15], of which 85 (46%) were male, 3 subjects (1.6%) used VKAs, and mean age was 61±6.5 years. COVID-19 and control subjects in which use of VKAs was unknown were excluded from analysis.

Patients were followed 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 mechanical ventilation or died.

Radiologic features on admission

Severity of COVID-19 on computed tomography (CT) was semi-quantitively scored. Pulmonary involvement of each lobe was scored as follows: no involvement: 0, <5% involvement: 1, 5-25% involvement: 2, 26-49% involvement: 4, 50-75% involvement: 5,

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>75% involvement: 5. The sum of the score of all five lobes represents the total lung involvement and ranges from 0 (no involvement) to 25 (complete involvement) [16].

Quantification of vitamin K status

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). In brief, 50 µL of patient sample or calibrators are incubated with magnetic particles coated with murine monoclonal dpMGP antibody, an acridinium labelled murine monoclonal ucMGP antibody and assay buffer. The magnetic particles are captured using a magnet and a wash step performed to remove any unbound analyte. Trigger reagents are 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. Dp-ucMGP values below 300 pmol/L are considered to be in the normal healthy range. Assays were performed by Coagulation Profile BV, department of Biochemistry, Maastricht, The Netherlands.

Quantification of elastin degradation

Plasma (p) desmosine and isodesmosine (DES) levels were measured in a subgroup of 26 patients with COVID-19. pDES were used as biomarker for the rate of elastin degradation. DES are formed during the cross-linking of elastin precursors and are released in the bloodstream after degradation of mature elastin. pDES is therefore positively associated with the rate of systemic elastin degradation. DES fractions were measured using liquid chromatography-tandem mass spectrometry with deuterium-labelled desmosine as internal standard, as previously described [17,18]. 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

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210,000 ng/L. Assays were performed by Desmosine.com, Nijmegen, the Netherlands. For each pDES measurement in a COVID-19 patient, a virtual age-matched pDES value was calculated using published pDES equations: (50+2.91*age ng/L) [19].

Statistical analysis

Statistical analyses were performed using SPSS (version 24, IBM, Chicago, IL, USA). Continuous variables are presented as mean ± standard deviation (SD). Analysis of covariance (ANCOVA) was used to compare dp-ucMGP levels between COVID-19 patients and controls as well as to compare dp-ucMGP levels between COVID-19 patients with good and poor outcome, respectively. Both analyses were also performed after adjustment for age, gender and use of VKAs. In subjects with COVID-19, the correlation between dp-ucMGP and pDES was assessed using Pearson's correlation coefficient. For the association of dp-ucMGP and COVID-19 severity score on CT, Spearman's correlation coefficient was used. Continuous variables with a log-normal distribution were log-transformed before analyses. A p-value of <0.05 was used as threshold for statistical significance.

Results

Dp-ucMGP levels had a log-normal distribution and were log-transformed prior to analysis. Dp-ucMGP levels were significantly higher in COVID-19 patients (1,673±1,584 pmol/L) compared to healthy controls (536±291 pmol/L, p<0.0005, *figure 1*). Dp-ucMGP remained significantly higher in COVID-19 patients after correction for age, gender and use of VKAs (p<0.0005). Dp-ucMGP levels were significantly lower in COVID-19 patients with good outcome (1,299±1,056 pmol/L) compared to those with poor outcome (2,087±1,940 pmol/L; p<0.0005). Significance was maintained after correction for age, gender and use of VKAs (p=0.005).

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In COVID-19 patients, dp-ucMGP levels were significantly associated with disease severity on CT (r=0.25; p=0.014; *figure 2*).

pDES levels had a log-normal distribution and were log-transformed before analysis. pDES levels were significantly higher in COVID-19 patients (607±454 ng/L) compared to age-dependent reference values (254±42 ng/L; p<0.0005) [19]. In subjects with COVID-19, dp-ucMGP and pDES were significantly and positively correlated (r=0.65; p<0.0005; *figure 3*).

Discussion

We report high dp-ucMGP in severe COVID-19 – a biomarker reflecting poor vitamin K status – as well as a correlation between this reduced vitamin K status and poor outcome and accelerated elastic fiber degradation.

Although technically feasible [20], 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 the method recommended by most experts, presenting the availability of both vitamin K1 and K2 in the system. Based on the upregulation of MGP in IPF lungs [21], we considered dp-uc MGP (*i.e.* inactive MGP) as the most appropriate surrogate marker of total vitamin K status for our study in COVID-19 patients. Dp-ucMGP levels are inversely correlated with vitamin K status. Thus, subjects with high dp-ucMGP levels have low vitamin K status and *vice versa*. Measuring levels of dp-ucMGP has previously been proven to be a useful method to assess vitamin K status [22]. Dp-ucMGP inversely relates to mortality and other clinical meaningful endpoints in various cohorts [12,15,23-30]. Supplementation of vitamin K has a reducing effect on dp-ucMGP levels [22,

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31], and the opposite holds true with regard of the use of VKAs [22,32-34]. Furthermore, vitamin K administration decelerated progression of aortic valve calcification [35], arterial stiffness [36], and bone loss [37]. We found very high levels of dp-ucMGP in COVID-19 patients with poor prognosis. It might be tempting to speculate that vitamin K administration has an improving effect on vitamin K status in severe COVID-19 patients, however, this has never been studied in this patient group. Additionally, whether improving vitamin K status would correlate with better prognosis in SARS-CoV-2-infected individuals has to be tested.

Low dietary vitamin K intake and use of VKAs are evident causes of vitamin K deficiency. Accelerated consumption of vitamin K may also be a potential reason for vitamin K deficiency in patients with severe COVID-19, as previously suggested for chronic obstructive pulmonary disease (COPD) [38].

Vitamin K-activated MGP is generally accepted as an inhibitor of vascular calcification [39]. There are scientific leads suggesting that MGP also plays a role in the pathogenesis of lung fibrosis [21,40]. MGP is crucial for the protection of elastic fibers against mineralization, given that other calcification inhibitors – such as fetuin – are too large to enter the interior of these fibers [39,41]. Elastic fibers are essential components of the extracellular matrix in dynamic tissues such as lungs and prone to mineralization. This is exemplified by vascular calcification characteristically starting in elastic fibers of arterial walls [42,43]. Degradation, fibrosis and mineralization of the extracellular matrix are interrelated pathomechanistic processes [42], as synthesis of matrix metalloproteinases (MMPs) and transforming growth factor β (TGF-β) enhances in parallel with elastic fiber calcification [44], and as partially degraded elastic fibers are more prone to mineralization due to increased polarity [45]. TGF-β is regarded as the master regulator of fibrosis, and massive fibrosis may be present in lungs of patients with severe COVID-19 [46]. MMPs are key proteases, and some of them have the

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ability to degrade elastic fibers. We measured higher circulating levels of DES in COVID-19 patients than would be expected in age-matched controls [19], indicating enhanced proteolytic activity in COVID-19. Vitamin K deficiency induced by administration of VKAs caused both elastic fiber degradation and calcification in an animal model [43]. Remarkably, upregulation of MMP synthesis preceded overt mineralization [43]. In the present study, an inverse correlation was found between vitamin K status and pDES in COVID-19 patients, suggesting a pathomechanistic link between vitamin K deficiency and accelerated elastolysis. This relationship has previously also been demonstrated in patients with COPD [15] and may be an indication for a link between vitamin K deficiency, insufficient MGP carboxylation and aberrant tissue remodeling in patients with COVID-19.

SARS-CoV-2 has the ability to enter human cells through binding with its spike proteins to angiotensin-converting enzyme 2 (ACE2) on outer cell surfaces [47]. Synthesis of cytokines is upregulated by infected cells upon cell entry of SARS-CoV-2 [48,49]. Proinflammatory mediators recruit immune cells and increase pulmonary permeability [50,51]. Extensive interstitial inflammatory infiltrates are typically seen in lungs of patients with COVID-19-induced ARDS [52]. Decreased integrity of the alveolar-capillary membrane and consequent pulmonary edema are hallmark features of ARDS and probably also of COVID-19-related lung disease [53], given the extensive ground glass abnormalities that are typically seen on chest computed tomography in COVID-19 as well as the feeling of drowning that these patients frequently describe [54,55].

Protein C and S are two vitamin K-dependent plasma proteins that work in concert as a natural anticoagulant system. There are several mechanistic steps in the pathogenesis of COVID-19 on which protein C and S may have an effect. Protein S has the ability to ameliorate increase of proinflammatory cytokines [56], and protein C also has anti-inflammatory properties [57]. Alveolar epithelial type 2 cells are particularly affected by

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SARS-CoV-2 [58], and protein S may have a protective effect against alveolar epithelial cell apoptosis [56].

Inflammation and coagulation are significantly interrelated. Cytokines leak to the circulation where they contribute to systemic coagulation by upregulating procoagulant and downregulating anticoagulant activity [59]. This may be a plausible explanation for the high incidence of coagulopathy and venous thromboembolism in patients with severe COVID-19 [3,4]. Reduced integrity of the alveolar-capillary barrier may also lead to a pulmonary procoagulant state due to influx of coagulation factors from the circulation into the lungs, which may have contributed to hemorrhagic infarction and necrosis in lungs of a non-surviving COVID-19 patient [46]. Whereas thrombin promotes endothelial cell barrier dysfunction [60], protein C may be protective against disruption of the alveolar-capillary membrane [61]. We hypothesize that concomitant administration of LMWH and vitamin K may be of benefit in patients with COVID-19 to inhibit coagulation and stimulate anticoagulation, which should be tested in a clinical trial.

Major strengths of our study are the use of an objective biomarker to quantify vitamin K status and the proposal of a potential disease mechanism that might underlie our findings. However, there were also some limitations that should be addressed. Since dp-ucMGP levels are increased in comorbidities that are related to poor outcome in COVID-19 [12-14], we are unable to determine whether deficiency of vitamin K predisposes to the development of severe COVID-19 or is merely an epiphenomenon. Furthermore, we included COVID-19 patients from a single hospital. Our findings therefore need confirmation in patient cohorts from different centers before drawing any definite conclusions. Another limitation is that we had to make use of a historical control group, due to the implementation of quarantines and social distancing practices to contain the pandemic. We do not consider this to be a major problem, however, as differences between historical controls and COVID-19 patients were of

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such a magnitude that loss of significance when comparing to another control group would be unlikely.

In conclusion, vitamin K status was reduced in COVID-19 patients compared to controls and was associated with disease severity. Preliminary evidence was provided suggesting a potential mechanistic link between reduced vitamin K status and accelerated tissue degradation. 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 designed the study. LJS, PL, and CM were responsible for the dp-ucMGP and JMWO for the desmosine measurements. JW and EGAK analyzed the data, and IP performed the statistical analysis. RJ, IP, and JW wrote the first draft of the manuscript. ASMD, LJS, JMWO, TMH, and EFMW critically revised the manuscript.

Conflict of interest statements

LJS discloses consultancy to Immunodiagnostic systems (IDS) and research grants from Nattopharma not related to this research. JMWO and RJ are owners of Desmosine.com. RJ discloses a patent for copper-heparin inhalation therapy in emphysema (WO/2019/139479) and application of a patent for vitamin K status as a prognostic and therapeutic biomarker in COVID-19. ASMD, IP, JW, TMH, PL, CM, EGAK, and EFMW declare no competing interests.

Role of funding source

None

Ethics committee approval

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

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Tables and figures

Table 1.

	COVID-19		Controls
	Good outcome	Poor outcome	
Subjects	64	59	184
Age (years)	64±13	72±9.8	61±6.5
Male (%)	41 (64)	46 (77)	85 (46)
VKA use (%)	4 (6.3)	7 (12)	3 (1.6)
Hypertension (%)	27 (42)	22 (37)	41 (22)
Diabetes mellitus (%)	14 (22)	14 (24)	6 (3.3)
Cardiac or cardiovascular disease (%)	16 (25)	20 (34)	10 (5.4)
Asthma/COPD (%)	13 (20)	12 (20)	0 (0)
Other respiratory disease (%)	5 (7.8)	8 (14)	-
Immunocompromised (%)	4 (6.3)	2 (3.4)	-
Dialysis dependent (%)*	1 (1.6)	2 (3.4)	-
Active malignancy (%)	5 (7.8)	6 (10)	0 (0)

COVID-19: Coronavirus 2019; *VKA*: Vitamin K antagonist; *COPD*: chronic obstructive pulmonary disease

* At admission

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Figure 1.

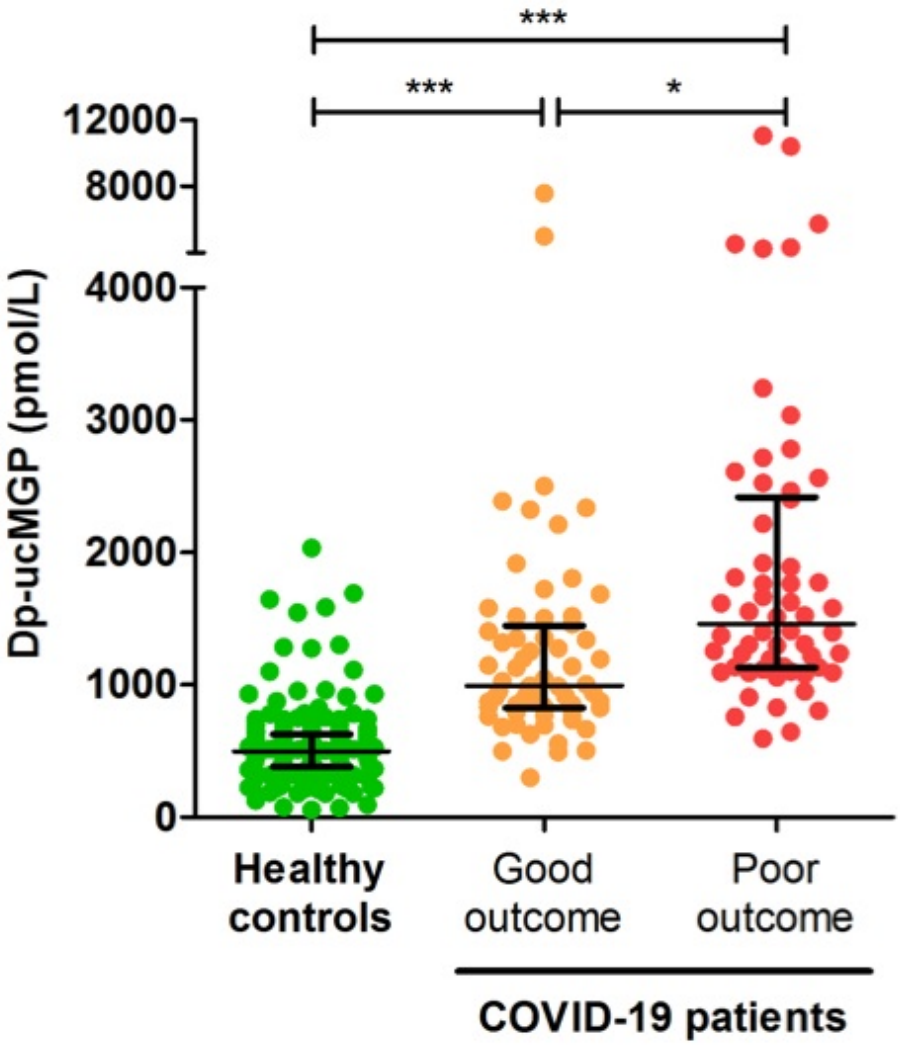

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Figure 2.

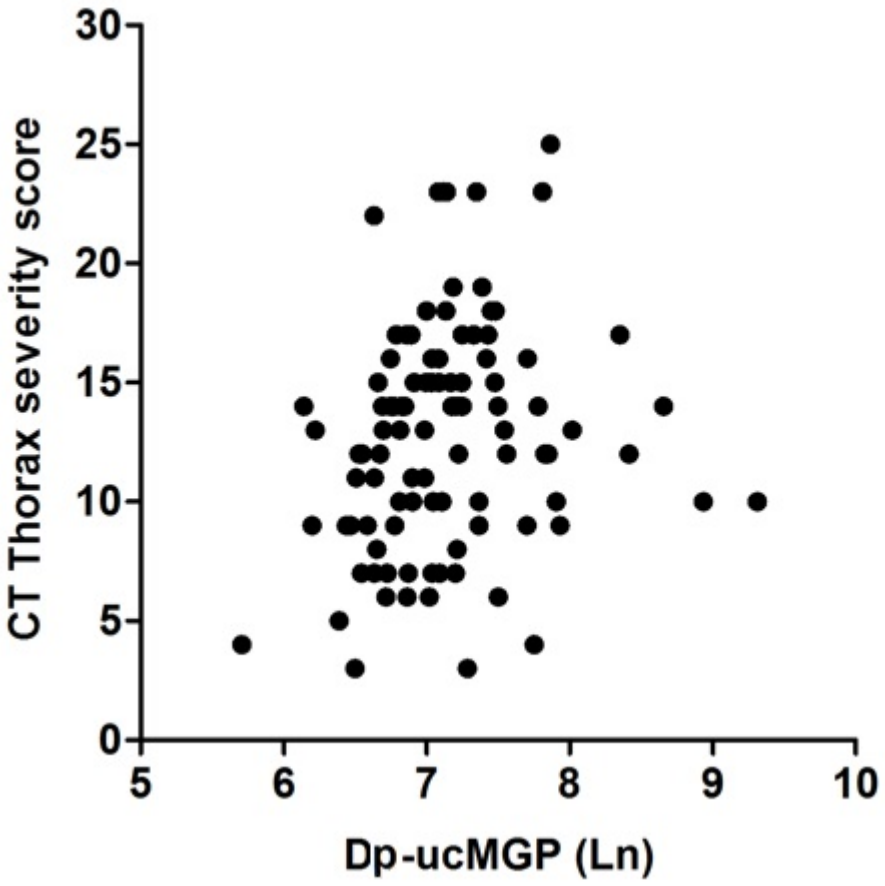

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Figure 3.