Quantitative MRI in obesity & reno-cardiovascular function

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Determinants of Impaired Renal and Vascular Function are associated with higher Levels of Procoagulant Factors in the General Population

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ABSTRACT

Background

Impaired renal and vascular function have been associated with venous thrombosis, but the mechanism is unclear.

Objectives

We investigated whether estimated glomerular filtration rate (eGFR), urinary albumin-creatinine ratio (UACR), and pulse wave velocity (PWV) are associated with a procoagulant state.

Methods

In this cross-sectional analysis of the NEO Study, eGFR, UACR, fibrinogen, and coagulation factors (F)VIII, FIX, and FXI were determined in all participants (n=6,536), and PWV was assessed in a random subset (n=2,433). eGFR, UACR and PWV were analysed continuously and per percentile; per 6 categories for eGFR (>50th[reference] to <1st) and UACR (<50th[reference], to >99th), and per 4 categories (<50th[reference] to >95th percentile) for PWV. Linear regression was used and adjusted for age, sex, total body fat, smoking, education, ethnicity, total cholesterol, CRP, and vitamin K antagonists use (FIX).

Results

Mean age was 55.6 years, mean eGFR 86.0 ± 12 ml/1.73m² and median UACR 0.4 mg/mmol (25th,75th percentile 0.3;0.7). All coagulation factors showed a procoagulant shift with lower renal function and albuminuria. For example, FVIII was 22 IU/dL (95% CI 13, 32) higher in the eGFR <1st percentile compared with >50th percentile, and FVIII was 12 IU/dL (95% CI 3, 22) higher in the UACR >99th percentile compared with <50th percentile. PWV was positively associated with coagulation factors FIX and FXI in continuous analysis, per m/s difference in PWV, FIX was 2.0 IU/dL (95% CI 0.70, 3.2) higher.

Conclusions

Impaired renal and vascular function was associated with higher levels of coagulation factors, underlining the role of renal function and vascular function in the development of venous thrombosis.

INTRODUCTION

Venous thrombosis plays a major role in the morbidity and mortality of patients with advanced renal disease (1). Previous studies have shown that impaired renal function and the presence of microalbuminuria in patients with Chronic Kidney Disease (CKD) are associated with an increased risk of venous thrombosis (2, 3). To illustrate, individuals with an estimated glomerular filtration rate (eGFR) <60 ml/min/1.73m² have a 2-3-fold increased risk of venous thrombosis compared with individuals with normal renal function (4, 5). Moreover, impaired renal function is associated with higher levels of coagulation factors including fibrinogen, factors (F)VII, FVIII, FIX, FXI, and von Willebrand factor (6). This supports the hypothesis that the increased risk of venous thrombosis in CKD could be induced by elevated coagulation factors levels due to generalized endothelial damage (6).

Previous studies have focused on either the increased risk of venous thrombosis, or the presence of CKD in relation to coagulation factors (5). However, it remains unclear whether impaired vascular function is associated with higher levels of coagulation factors in the general population. Moreover, the few studies thus far evaluating associations between renal function, albuminuria and coagulation did not account for inflammation as a potential confounder (7, 8). Atherosclerosis measured by intima-media thickness has been suggested as a possible risk factor of VTE (9), and atherosclerosis has been associated with a procoagulant state (10). Arterial stiffness assessed by pulse wave velocity (PWV) is a functional and more robust marker for early atherosclerosis than intima-media thickness (11, 12), and has been linked with endothelial shear stress (13). Moreover, recent insights showed that arterial stiffness is regulated by hemodynamic and mechanosensitive properties of endothelial cells reflecting systemic vascular function (14, 15). Thus far, the association between vascular function (i.e. endothelial dysfunction, and arterial stiffness) and haemostasis in the general population remains elusive.

We hypothesize that impaired renal function and impaired vascular function is associated with hypercoagulability, evidenced by a procoagulant shift in coagulation factors which could ultimately lead to venous thrombosis, (**Fig. 1**).

MATERIAL AND METHODS

Study population and study design

The Netherlands Epidemiology of Obesity (NEO) study is a population-based, prospective cohort study in 6,671 individuals aged 45–65 years (16). Recruitment of participants started in September 2008 and was completed at the end of September 2012. Men and women living in the greater area of Leiden (in the West of the Netherlands) were invited by letters sent by GPs and municipalities and by local advertisements. They were invited

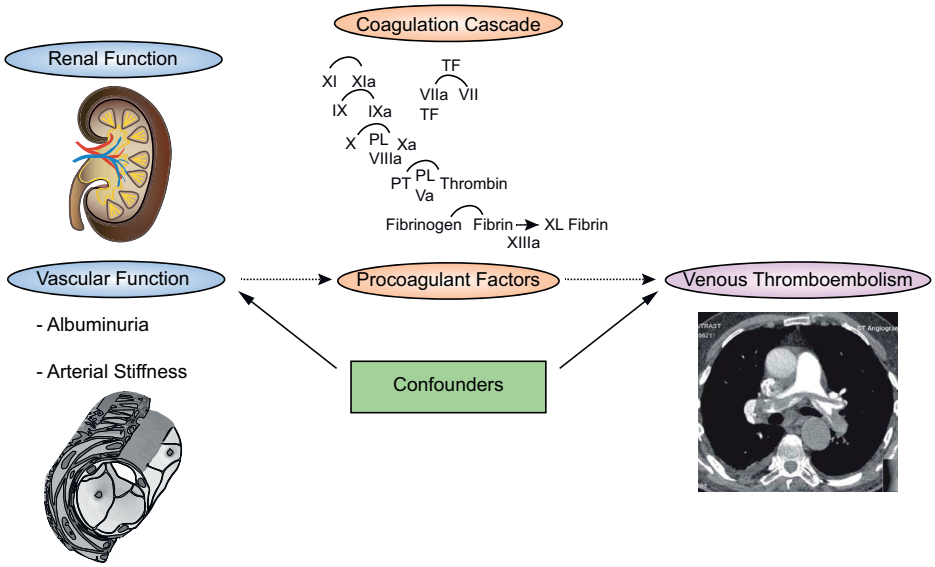

Figure 1. Hypothesis pathway of presumed effects of renal and vascular function (albuminuria and arterial stiffness) on prothrombotic coagulation factors and the subsequent development of venous thromboembolic events.

to respond if they were aged between 45 and 65 years and had a self-reported body mass index (BMI) of 27 kg/m^2 or higher. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited to participate irrespective of their BMI, allowing for a reference distribution of BMI. Exclusion criteria were missing data on eGFR, urinary albumin excretion, fibrinogen or FVIII, FIX, or FXI. All participants were screened for potential contraindications for MR imaging (metallic devices, claustrophobia or a body circumference of ≥1.70 m) at the NEO study center, and approximately 30% of the of the participants eligible for MR imaging were randomly selected to undergo MRI imaging. Participants with missing PWV measurements due to technical failures, were excluded for further analysis. The Medical Ethical Committee of the Leiden University Medical Center (LUMC) approved the design of the study and all participants gave their written informed consent.

Data collection

Participants were invited to a baseline visit at the NEO study center of the LUMC after an overnight fast. Prior to this study visit, participants collected their urine over 24 h and completed a general questionnaire at home to report demographic, lifestyle and clinical information. The participants were asked to bring all medication they were using to the study visit. At the baseline visit all participants underwent physical examination including anthropometry and blood pressure. Height was measured using a calibrated tape

measure and body weight and percentage of total body fat (TBF) were measured using the Tanita bio impedance balance (TBF-310, Tanita International Division, UK). Weight in kilograms was divided by height is meters squared to calculate BMI. Fasting blood samples were drawn from the antecubital vein after 5 min rest of the participant. Subsequently, fasting glucose, triglyceride, low- and high-density lipoprotein concentrations, C-reactive protein (CRP), serum creatinine, were measured with standard methods in the central clinical chemistry laboratory of the LUMC (16). Serum creatinine (mg/dl) was used to calculate estimated Glomerular Filtration Rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (17). Blood samples for coagulation factor measurements were drawn into tubes containing 0.106M trisodium citrate (Sarstedt, Nümbrecht, Germany). Plasma was obtained by centrifugation at 2500g for 10 min at room temperature and stored in aliquots at -80°C until testing. Fibrinogen activity was measured according to the method of Clauss. Activity of FVIII, FIX, and FXI were measured with a mechanical clot detection method on an ACL TOP 700 analyzer (Werfen, Barcelona, Spain). All assays were performed by laboratory technicians who were unaware of the status of the samples. Urinary albumin was measured in early morning single urine void using an immunoturbidimetric assay and creatinine using a Jaffe kinetic compensated method between September 1st, 2008 until November 30th, 2010 and an enzymatic assay (IDMS calibrated against SRM 967) since December 1st, 2010 until the end of the inclusion period. Because urinary creatinine concentrations are not affected by pseudochromogens they are exchangeable using either a Jaffe or an enzymatic method. Normal renal function was defined as eGFR>90 ml/min/1.73m², mildly to moderately reduced renal function as eGFR 45-90 ml/min/1.73m², and moderately to severely reduced renal function as eGFR <45 ml/min/1.73m² (18). Normal range albuminuria was defined as UACR <3 mg/mmol (30-300 mg/g), microalbuminuria (also referred to as moderately increased albuminuria) as UACR of 3-30 mg/mmol (30-300 mg/g), and albuminuria (also referred to as macroalbuminuria or severely increased albuminuria) as UACR >30 mg/mmol (18).

Assessment of arterial stiffness

For the assessment of arterial stiffness, pulse wave velocity (PWV) was determined on a 1.5 Tesla (T) whole-body magnetic resonance imaging (MRI) scanner (Gyroscan ACS/NT15, Philips, Best, The Netherlands) using multislice, two one-directional in-plane velocity-encoded MRI. PWV was calculated by the ratio of the distance along the aortic center line (Δx) and the transit-time of the propagating systolic pulse wave between two measurement sites (Δt) (proximal aorta and distal aorta summed) (19). An overview of the assessment of PWV is given in **Figure 2**.

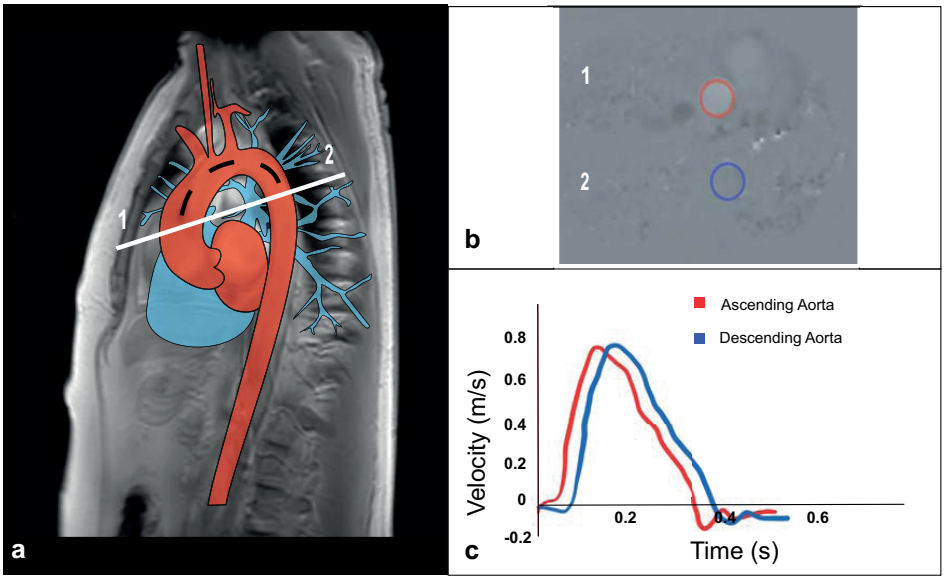

Figure 2. Assessment of arterial stiffness using MRI (a) MRI scout view of the thoracic aorta with anatomical drawing indicating proximal (1 ascending aorta) and distal (2 descending aorta) used for the planning of the velocity-encoded MRI planes. (b) Phase image of measurement site 1 and 2. (c) Derived velocity wave curves forms from measurement site 1 and 2.

Statistical analysis

In the NEO study individuals with a BMI of 27 kg/m² or higher were oversampled. First, inhabitants of Leiden and its surroundings between 45 and 65 years of age and with a self-reported BMI of 27 kg/m² or higher were invited to participate in the NEO study. In addition, we included a reference population. To that extent, all inhabitants between 45 and 65 years living in one municipality, Leiderdorp, were asked to participate irrespective of their BMI. This resulted in an additional sample of 1671 participants with a BMI distribution that was similar to the BMI distribution of the general Dutch population (20). If inference is made on the general population, the overrepresentation of overweight and obese participants in the NEO study may introduce bias, because of the skewed BMI distribution in the NEO population. Weighting towards the BMI distribution of the general population may solve this problem (21). Using the BMI distribution of the reference population, we calculated weight factors for the NEO population, resulting in a higher weight factor for participants with a lower BMI (**supplemental Table 3**). Use of sampling weights yield results which apply to a population-based study without oversampling of individuals with a high BMI (22). **Supplementary Figure 1** shows that the use of the weight factors results in a BMI distribution that is similar to that of the general population. Baseline characteristics of the weighted study population are expressed as mean (SD), median (25^{th} and 75^{th} percentile) or as percentage. Determinants were eGFR (CKD-

EPI), urinary albumin-creatinine ratio (UACR), and pulse wave velocity (PWV). Outcome variables were fibrinogen, FVIII, FIX, and FXI. UACR was log-2 transformed since this variable has a right-skewed distribution. Both renal function and albuminuria were grouped into six categories based on percentiles (>50th [reference], 10th to 50th, 5th to 10th, 2.5th to 5th, 1st to 2.5th, and <1st percentile for eGFR) and (<50th [reference], 50th to 90th, 90th to 95th, 95th to 97.5th, 97.5th to 99th, and >99th percentile for albuminuria). Because of the smaller sample size, pulse wave velocity was grouped into four instead of six categories based on percentiles (<50th [reference], 50th to 90th, 90th to 95th, >95th percentile). The use of percentile groups allows for the evaluation of wide ranges of values, particularly for abnormal levels. We calculated age- and sex-adjusted mean differences with 95% confidence intervals (CIs) in levels of hemostatic factors for the defined categories, compared with the reference using linear regression. Multiple linear regression was used to calculate the difference with 95% CIs in levels of hemostatic factors for every 10 ml/min/1.73m² change in eGFR, per two-fold change in albuminuria, and per ml/min change in PWV. Crude analyses were adjusted for age, sex, smoking (current, former, never), total body fat, CRP, total cholesterol, use of vitamin K antagonists (for factor IX only), ethnicity, and education. In order to correct for dyslipidaemia we only added total cholesterol to the regression analysis and not other measures of dyslipidemia in order to diminish the chance of an overfit statistical model. Additionally, we performed an analysis for clinical cut-off points for renal function (mildly to moderately reduced renal function [eGFR 45-90 ml/min/1.73m²], and moderately to severely reduced renal function [eGFR <45 ml/min/1.73m²]) and albuminuria (UACR >3 mg/mmol). Because gender and age specific cut-off points for vascular dysfunction based on PWV measured by MRI are yet to be defined, we investigated associations of PWV with the clotting factors over the whole range of PWV as a continuous variable and in percentiles. First degree linear spline modelling was used for exploring non-linear associations between renal function, vascular function and coagulation factors. Piecewise linear function was used, meaning that the adjusted regression models were composed of linear segments based on equally spaced knots (quintiles) (23). Analyses were performed using STATA (Statacorp, College Station, Texas, USA, version 12.0).

RESULTS

Baseline characteristics

The total NEO study population consisted of 6,671 participants. After consecutive exclusion of participants with missing data on renal function (n=50), albuminuria (n=27), coagulation factors (n=57) or fibrinogen (n=1) the final study population consisted of 6536 participants with a mean age of 55.6 ± 6 years. Mean eGFR was 86.0 ± 12.4 ml/

min/1.73m², normal eGFR (>90 ml/min/1.73m²) was present in 42%, mildly to moderately reduced renal function (eGFR 45-90 ml/min/1.73m²) in 57%, and moderately to severely reduced renal function (<45 ml/min/1.73m²) in 0.3% of the participants. Median UACR was 0.4 mg/mmol (25th, 75th percentile; 0.3; 0.7) and median UAE was 3.6 mg/L (25th, 75th percentile; 3.0, 4.8). Microalbuminuria was present in 2.0% and albuminuria in 0.4% of the participants, 4% of the participants had diabetes. ACE inhibitors or angiotensin-II antagonists were used by 14% of the participants. Baseline characteristics of the included study population are presented in **Table 1**. Approximately 30% of the participants who were eligible for additional MRI, were randomly selected to undergo PWV measurement (n=2,576). Participants with missing PWV measurements due to technical failures (n=143), were excluded for further analysis. The complete subgroup of participants with available PWV measurements consisted of 2,433 individuals, and was not statistically different from participants without PWV measurements (data not shown).

Associations of determinants of vascular function with coagulation factors

Adjusted mean differences of renal function, albuminuria, and arterial stiffness percentiles, and coagulant factors are provided in **Tables 2-5**. Crude mean differences and linear regression coefficients are provided in the **Supplemental Table 1 and 2**.

Compared with participants with an eGFR >50th percentile, the adjusted mean coagulation factor levels in participants with an eGFR <1st percentile were 33.3 (95% CI 13.0, 53.5) mg/dL for fibrinogen, 22 (95% CI 13, 32) IU/dL for FVIII, 16 (95% CI 7, 24) IU/dL for FIX and 4 (95% CI -5, 14) IU/dL for FXI. Similar results were found when clinical cut-off points were used to categorize renal function instead of percentiles. Participants with moderately to severely decreased kidney function (eGFR <45 ml/min/1.73m²) compared to participants with normal renal function (eGFR >90 mL/min; Table 5), had adjusted mean differences of 44.5 (95% CI 9.5, 79.4) mg/dL for fibrinogen, 43 (95% CI 22, 64) IU/dL for FVIII, 25 (95% CI 5, 46) IU/dL for FIX, and 2 (95% CI -1, 5) IU/dL for FXI.

For albuminuria the adjusted mean coagulation factor levels in participants with an UACR >99th percentile were 12.3 (95% CI -2.6, 27.10 mg/dL for fibrinogen, 12 (95% CI 3, 22) IU/dL for FVIII, 9 (95% CI 3, 14) IU/dL for FIX, and 4 (95% CI 0, 9) IU/dL for FXI compared with participants with an UACR <50th percentile. When using clinical cut-off points to categorize albuminuria instead of percentiles, participants with micro- and/or macroalbuminuria (UACR >3 mg/mmol) had as compared with participants with normal range albuminuria (UACR <3 mg/mmol) (**Table 5**), adjusted mean differences of 5.9 (95% CI -3.8, 15.3) mg/dL for fibrinogen, 2 (95% CI -6, 9) IU/dL for FVIII, 3 (95% CI -1, 6) IU/dL for FIX, and 2 (95% CI -1, 5) IU/dL for FXI.

Table 1. Characteristics of the study population (n=6,536)

Characteristic	
Demographic/anthropometric	
Age (y)	55.6 (6)
Sex (% men)	48
Ethnicity (% white)	95
Education (% higher)	46
BMI (kg/m^2)	26 (4)
Tobacco smoking (% never)	38
Comorbidity	
Hypertension (% yes)	34
Diabetes* (% yes)	4
Cardiovascular disease† (% yes)	6
Thromboembolic event‡ (% yes)	0.2
Medication use	
Antihypertensives (% yes)	24
Glucose lowering medication (% yes)	2.7
Vitamin K antagonist (% yes)	1.3
Lipid lowering drug (% yes)	11
ACE/Angiotensin-II antagonists (% yes)	14
Blood pressure	
Systolic (mmHg)	130 (17)
Diastolic (mmHg)	83 (10)
Mean arterial pressure (mmHg)	99 (12)
Biomarkers	
Serum creatinine (umol/L)	77.4 (15)
eGFR CKD-epi (ml/min/1.73m^2)	86.0 (12.4)
UAE (mg/l)	3.6 (3.0; 4.8)
UACR (mg/mmol)	0.4 (0.3; 0.7)
Total cholesterol (mmol/L)	5.7 (1.1)
Triglycerides (mmol/L)	1.2 (0.8)
High-density lipoprotein (mmol/L)	1.6 (0.5)
Low- density lipoprotein (mmol/L)	3.6 (1.0)
Fasting glucose (mmol/l)	5.4 (1.0)
C-Reactive Protein (mg/L)	1.9 (2.8)
Arterial Stiffness	
Pulse wave velocity§ (m/s)	6.6 (1.3)
Coagulation factors	
Fibrinogen (g/L)	290 (56)
Factor VIII (IU/dL)	122 (33)
Factor IX (IU/dL)	115 (21)
Factor XI (IU/dL)	116 (19)

Results were weighted toward the BMI distribution of the general population (n=6,536). Results are expressed as %, means (standard deviation) or medians (25th, 75th percentile). Diabetes*; raised fasting plasma glucose concentrations (≥5.56 mmol/L) or on drug treatment to lower glucose concentrations. Cardiovascular disease†; myocardial infarction, angina pectoris, congestive heart failure, Thromboembolic event‡; pulmonary embolism, deep venous thrombosis. eGFR, estimated glomerular filtration rate according to the CKD-EPI formula; UAE, urinary albumin excretion; UACR, urinary albumin-creatinine ratio. Pulse wave velocity§; assed in n=2,433

Table 2. Regression between eGFR percentiles and coagulant factors

	Adjusted* mean difference (95% CI) compared with >50th percentile (eGFR >87.3mL/min/1.73m²)				
Coagulation Factor	50th-10th Percentile (eGFR ranges, 69.4-87.3 mL/min/1.73m²)	5th-10th Percentile (eGFR ranges, 65.3-69.4 mL/min/1.73m²)	2.5th-5th Percentile (eGFR ranges, 60.5-65.3 mL/min/1.73m²)	1th-2.5th Percentile (eGFR ranges, 55.3-60.5 mL/min/1.73m²)	<1st Percentile (eGFR <55.3 mL/min/1.73m²)
Fibrinogen (g/L)	-0.4 (-3.7, 3.0)	-5.3 (-11.1, 0.5)	-1.1 (-12.0, 9.8)	0.9 (-11.4, 13.1)	33.3 (13.0, 53.5)
Factor VIII (IU/dL)	3 (0, 5)	3 (-2, 7)	11 (3, 19)	9 (-1, 20)	22 (13, 32)
Factor IX (IU/dL)†	1 (-1, 2)	5 (2, 8)	4 (1, 8)	5 (0, 10)	16 (7, 24)
Factor XI (IU/dL)	0 (-1, 2)	2 (-1, 5)	-1 (-5, 3)	4 (-3, 10)	4 (-5, 14)

Results were weighted toward the BMI distribution of the general population (n=6,536). *Adjusted for; sex, age, smoking, total body fat, CRP, total cholesterol, ethnicity and education. †Vitamin K antagonist users were excluded from the analysis. Corresponding linear regression coefficients, 95% Confidence Intervals.

Table 3. Regression between UACR percentiles and coagulant factors

	Adjusted* mean difference (95% CI) compared with <50th percentile (UACR <0.49 mg/mmol)				
Coagulation Factor	50th-90th Percentile (UACR ranges, 0.49-1.11 mg/mmol)	90th-95th Percentile (UACR ranges, 1.11-1.64 mg/mmol)	95th-97.5th Percentile (UACR ranges, 1.64-2.96 mg/mmol)	97.5th-99th Percentile (UACR ranges, 2.96-6.21 mg/mmol)	>99th Percentile (UACR >6.21 mg/mmol)
Fibrinogen (g/L)	1.3 (-2.4, 5.0)	2.1 (-4.7, 8.9)	5.5 (-5.1, 16.1)	0.8 (-11.1, 12.7)	12.3 (-2.6, 27.1)
Factor VIII (IU/dL)	-4 (-6, -1)	-5 (-10, 0)	-3 (-10, 4)	-8 (-18, 1)	12 (3, 22)
Factor IX (IU/dL)†	0 (-2, 1)	1 (-2, 4)	0 (-4, 4)	-2 (-5, 2)	9 (3, 14)
Factor XI (IU/dL)	1 (-1, 2)	-2 (-5, 1)	-2 (-6, 2)	1 (-3, 5)	4 (0, 9)

Results were weighted toward the BMI distribution of the general population (n=6,536). *Adjusted for; sex, age, smoking, total body fat, CRP, total cholesterol, ethnicity and education. †Vitamin K antagonist users were excluded from the analysis. Corresponding linear regression coefficients, 95% Confidence Intervals.

Table 4. Regression between PWV percentiles and coagulant factors

	Adjusted* mean difference (95% CI) compared with <50th percentile (PWV <6.31 m/s)		
Coagulation Factor	50th-90th Percentile (PWV ranges, 6.31-8.32 m/s)	90th-95th Percentile (PWV ranges, 8.31-9.14 m/s)	>95th Percentile (PWV >9.14 m/s)
Fibrinogen (g/L)	1.4 (-4.6, 7.4)	-6.8 (-15.7, 2.1)	11.4 (-2.6, 25.4)
Factor VIII (IU/dL)	0 (-4, 4)	1 (-6, 8)	-1 (-9, 8)
Factor IX (IU/dL) †	3 (1, 5)	9 (5, 13)	7 (-1, 15)
Factor XI (IU/dL)	3 (0, 5)	3 (-1, 8)	4 (-2, 10)

Results were weighted toward the BMI distribution of the general population (n=2,433). *Adjusted for; sex, age, smoking, total body fat, CRP, total cholesterol, ethnicity and education. †Vitamin K antagonist users were excluded from the analysis. Corresponding linear regression coefficients, 95% Confidence Intervals.

Table 5. Regression between clinical stages of eGFR, UACR and coagulant factors

	Adjusted* mean difference (95% CI) compared with normal kidney function (eGFR >90ml/min/1.73m²; 42.3%)		Adjusted* mean difference (95% CI) compared with normal UACR (<3 mg/mmol; 97.6%)
Coagulation Factor	eGFR 45-90 ml/ min/1.73m² (n=57.5%)	eGFR <45 ml/min/1.73m² (n=0.26%)	UACR >3 mg/mmol (n=0.24%)
Fibrinogen (g/L)	-1.4 (-4.8, 1.9)	44.5 (9.5, 79.4)	5.9 (-3.6, 15.3)
Factor VIII (IU/dL)	5 (3, 7)	43 (22, 64)	2 (-6, 9)
Factor IX (IU/dL) †	2 (0, 3)	25 (5, 46)	3 (-1, 6)
Factor XI (IU/dL)	0 (-1, 2)	21 (4, 38)	2 (-1, 5)

Results were weighted toward the BMI distribution of the general population (n=6,536). *Adjusted for; sex, age, smoking, total body fat, CRP, total cholesterol, ethnicity and education. †Vitamin K antagonist users were excluded from the analysis. Corresponding linear regression coefficients, 95% Confidence Intervals.

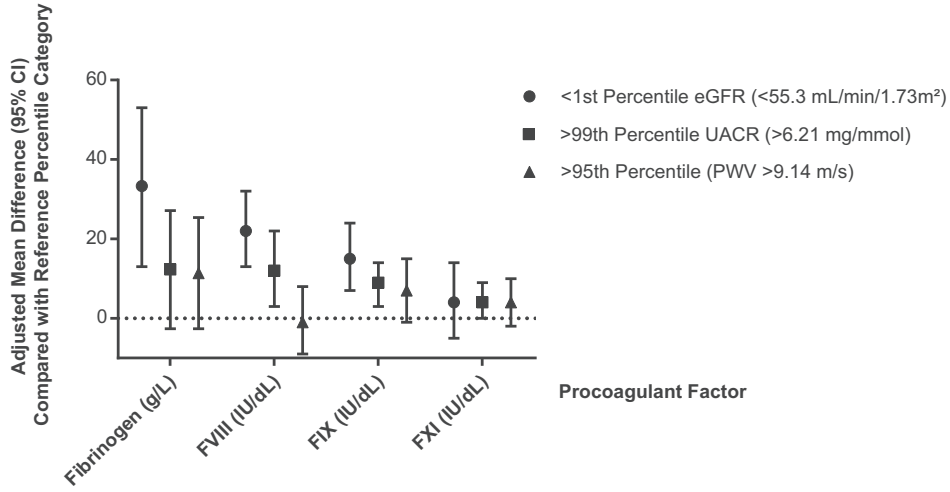

Figure 3. Overview of the adjusted mean factor levels of the <1st percentile eGFR, >99th percentile UACR and >95th percentile PWV compared with the reference percentile category. Results were weighted toward the BMI distribution of the general population (n=6,536) Adjusted for; sex, age, smoking, total body fat, CRP, total cholesterol, ethnicity and education Corresponding linear regression coefficients, 95% Confidence Intervals. The reference percentile category for eGFR was >50th percentile for eGFR, <50th percentile for UACR and PWV. Vitamin K antagonist users were excluded from the analysis of FIX.

For arterial stiffness the adjusted mean coagulation factor levels of participants with PWV >95th percentile were 11.4 (95% CI -2.6, 25.4) mg/dL for fibrinogen, -1 (95% CI -9, 8) IU/dL for FVIII, 7 (95% CI -1, 15) for FIX and 4 (95% CI -2, 10) for FXI compared with participants with PWV <50th percentile. An overview of the adjusted mean factor levels of the <1st percentile eGFR, >99th percentile UACR and >95th percentile PWV compared with the reference percentile category (>50th percentile for eGFR and <50th percentile

for UACR and PWV) is visualized in **Figure 3**. Spline models showing the association between eGFR, UACR, PWV and coagulation factors fibrinogen, FVIII, FIX, and FXI, suggest an inverse relationship between renal function and coagulation factors, and a positive relationship between vascular function and coagulation factors (except for fibrinogen). Results of spline models are visualized in **Supplementary Figure 2**.

DISCUSSION

In this large population-based cross-sectional study, coagulation factors showed a procoagulant shift with all three determinants of lower vascular function, and found associations were most pronounced for factor VIII (for eGFR and UACR) and factor IX (for PWV).

We found that the adjusted mean coagulation factor levels for participants with an eGFR <60 ml/min/1.73m² were significantly higher for fibrinogen, FVIII, FIX, and FXI compared to participants with normal renal function (eGFR >90 mL/min), while controlling for various confounding factors including CRP. These findings are supported by results based on linear spline models except for the relationship between PWV and fibrinogen for which we have no biological explanation. Our study adds novel information to the previous population-based multi-ethnic MESA study that observed that a decline in renal function was associated with both coagulation factors (fibrinogen and FVIII), and an increase in inflammatory markers (such as CRP and IL-6) in participants without cardiovascular disease or chronic kidney disease (7, 24). However, previous observations were limited by the use of coagulation factors that are also acute phase reactant proteins, meaning that previous findings could have been solely a reflection of the association of endothelial dysfunction with inflammation.

In the context of CKD, markers of endothelial dysfunction have been associated with a hypercoagulable state, and raised levels of FVIII and von Willebrand factor with an increased thrombo-embolic risk (6, 25). This is supported by the observed normalization of endothelial dysfunction and hypercoagulability after renal transplantation (26, 27), suggesting a causal relationship between renal vascular function and hypercoagulability (28). We observed that higher levels of albuminuria associate with higher levels of coagulation factors. For the >99th UACR percentile (>6.21 mg/mmol) these findings were most pronounced for FVIII and fibrinogen. FVIII is of particular interest, since it is synthesized by vascular, glomerular, and tubular endothelium, as well as the sinusoidal cells of the liver. FVIII could therefore be considered both as a marker of endothelial dysfunction, and a risk factor for venous thrombosis (25). The found link between higher levels of albuminuria and a procoagulant state in this study, might be explained by endothelial regulation of vascular homeostasis, which could become dysfunctional due to sustained inflammatory endothelial activation (29). Two other studies evaluating the association

between UACR and venous thromboembolism, however did not observe such an association (30, 31). In the present study, the differences in adjusted mean coagulation factor levels for the highest / lowest percentile compared with the reference category was also most pronounced for eGFR, rather than for albuminuria or arterial stiffness.

In the current study we found a positive association between increased arterial stiffness and higher levels of FIX and FXI in continuous analysis, however this association was not convincingly present when the analyses were based on percentile cut-offs of arterial stiffness. Arterial stiffness is a reflection of the elastic properties of a vessel (i.e. compliance and ability to contract and dilate), which is mainly determined by the intima-media and can respond to mechanical changes through the production vasoactive molecules, extracellular matrix, and extracellular matrix-degrading proteases (15). Previous basic science studies have shown that mechanical injury to vasculature can result in increased procoagulant state (32), however further studies are needed to verify whether a true association exists between arterial stiffness and the coagulation system.

Strengths of our study are the large sample size with extensive phenotyping, including data on four different coagulation factors and data on three different measures of vascular function. Both albuminuria and arterial stiffness can be considered to reflect systemic vascular injury; albuminuria may potentially serve as a surrogate marker of systemic endothelial dysfunction (33), and arterial stiffness as a marker of intima-medial calcification (11, 34). Arterial stiffness was assessed in a large random subgroup using MRI, which is highly superior compared to conventional tonometry (19), and has not been studied in relation to coagulation thus far.

This study also has a number of limitations that need to be considered. The observed associations appeared to be the strongest in individuals with the lowest renal and vascular function. It must be noted that these individuals represented only 1% of the total population, because this is a population-based analysis without any prior selection criteria for chronic kidney disease. Therefore, our findings are representative for the general population rather than CKD patients. The results from the spline models also suggest an inverse relation between renal function and increased levels of procoagulant factors. Although our results are based on a population-based study, we cannot completely exclude the influence of other potentially clinically relevant conditions that would affect renal and vascular function in relation to coagulation factor levels. Another limitation was the use of estimated GFR rather than (invasive) gold standard exogenous clearance measurements. Although the CKD-EPI formula has proven to be more accurate and precise than the older MDRD formula, it has not been validated above 90 ml/min/1.73m² (35). Furthermore, in the present study overall markers of hypercoagulability or natural coagulation inhibitors such as d-dimer and antithrombin were unfortunately not measured and could not be taken into account in the analyses. Our findings support the hypothesis that vascular function is a marker of hypercoagulability, however the cross-sectional observational

nature of our study precludes a causal interpretation. The exact mechanism explaining why CKD is associated with an increased risk of venous thrombosis remains unclear. Recently, potential novel pathways for the regulation of haemostasis and thrombosis in kidney disease have been described suggesting that a subclinical inflammatory state initiates glomerular injury via altered expression of coagulation factors, rather than impaired renal function or vascular damage initiating procoagulant changes (28). This has been supported by various laboratory findings and preclinical studies showing that renal cell-specific expression and activity of coagulation proteases, and its associated regulators and receptors tend to alter during renal disease processes (28). Future analysis with repeated measurements could contribute to the assessment of the direction of the observed associations between vascular function and coagulation factors.

In summary, in this middle-aged sample of the general population impaired renal and vascular function (i.e. albuminuria and arterial stiffness) was associated with higher levels of coagulation factors. These findings support the hypothesis that impaired renal and vascular function plays a role in the etiology of venous thrombosis.

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Supplemental Table 1. Regression between PWV, eGFR, UACR and coagulant factors

Coagulation Factor	Per 10 ml/min/1.73m² lower eGFR (n=6,536)	Per 2-fold higher UACR (mg/mmol) (n=6,536)	Per m/s higher PWV (n=2,433)
Fibrinogen (g/L)			
Crude	1.1 (-0.6, 2.7)	5.4 (3.7, 7.2)	2.3 (0.5, 4.0)
Adjusted	0.4 (-1.0, 1.9)	2.0 (0.6, 3.3)	0.6 (-1.9, 3.1)
Factor VIII (IU/dL)			
Crude	3.1 (2.2, 4.1)	0.4 (-0.6, 1.3)	1.4 (0.3, 2.4)
Adjusted	2.3 (1.3, 3.3)	-0.9 (-1.9, 0.1)	0.4 (-1.2, 1.9)
Factor IX (IU/dL)*			
Crude	1.1 (0.5, 1.7)	0.8 (0.2, 1.4)	1.9 (1.3, 2,5)
Adjusted	1.0 (0.4, 1.6)	0.3 (-0.2, 0.8)	2.0 (0.7, 3.2)
Factor XI (IU/dL)			
Crude	0.8 (0.1, 1.4)	1.0 (0.5, 1.6)	0.7 (0.1, 1.3)
Adjusted	0.5 (-0.1, 1.1)	-0.1 (-0.6, 0.5)	1.1 (0.1, 2.0)

Results were weighted toward the BMI distribution of the general population. *Vitamin K antagonist users were excluded from the analysis. Adjusted for; sex, age, smoking, total body fat, CRP, total cholesterol, ethnicity and education. Corresponding linear regression coefficients, 95% Confidence Intervals. Regression coefficients reflect the difference in coagulant factor per 10 ml/min/1.73m^2 eGFR, per 2-fold difference in UACR and per m/s difference in PWV.

Supplemental Table 2. Regression between eGFR, UACR and PWV percentiles and coagulant factors

	Crude and adjusted# mean difference (95% CI) compared with >50th percentile (eGFR >87.3mL/min/1.73m²)				
Coagulation Factor	50th-10th Percentile (eGFR ranges, 69.4-87.3 mL/min/1.73m²)	5th-10th Percentile (eGFR ranges, 65.3-69.4 mL/min/1.73m²)	2.5th-5th Percentile (eGFR ranges, 60.5-65.3 mL/min/1.73m²)	1th-2.5th Percentile (eGFR ranges, 55.3-60.5 mL/min/1.73m²)	<1st Percentile (eGFR <55.3 mL/min/1.73m²)
Fibrinogen (g/L)					
Crude	-2.7 (-6.4, 1.9)	-6.2 (-13.9, 1.6)	8.3 (-4.4, 21.0)	10.1 (-5.2, 25.4)	56.8 (31.1, 82.5)
Adjusted*	-0.4 (-3.7, 3.0)	-5.3 (-11.1, 0.5)	-1.1 (-12.0, 9.8)	0.9 (-11.4, 13.1)	33.3 (13.0, 53.5)
Factor VIII (IU/dL)					
Crude	4 (1, 6)	6 (0, 11)	15 (7, 23)	13 (3, 23)	31 (22, 40)
Adjusted*	3 (0, 5)	3 (-2, 7)	11 (3, 19)	9 (-1, 20)	22 (13, 32)
Factor IX (IU/dL)†					
Crude	0 (-1, 2)	4 (1, 8)	7 (2, 11)	6 (0, 12)	20 (10, 30)
Adjusted*	1 (-1, 2)	5 (2, 8)	4 (1, 8)	5 (-0, 10)	15 (7, 24)
Factor XI (IU/dL)					
Crude	1 (-1, 2)	3 (0, 6)	1 (-4, 6)	5 (-2, 12)	7 (-3, 18)
Adjusted*	0 (-1, 2)	2 (-1, 5)	-1 (-5, 3)	4 (-3, 10)	4 (-5, 14)

	Crude and adjusted# mean difference (95% CI) compared with <50th percentile (UACR <0.49 mg/mmol)				
Coagulation Factor	50th-90th Percentile (UACR ranges, 0.49-1.11 mg/mmol)	90th-95th Percentile (UACR ranges, 1.11-1.64 mg/mmol)	95th-97.5th Percentile (UACR ranges, 1.64-2.96 mg/mmol)	97.5th-99th Percentile (UACR ranges, 2.96-6.21 mg/mmol)	>99th Percentile UACR >6.21 mg/mmol)
Fibrinogen (g/L)					
Crude	6.4 (2.2, 10.6)	17.4 (7.8, 27.0)	24.1 (9.8, 38.5)	14.3 (-0.4, 29.0)	37.7 (16.8, 58.6)
Adjusted*	1.3 (-2.4, 5.0)	2.1 (-4.7, 8.9)	5.5 (-5.1, 16.1)	0.8 (-11.1, 12.7)	12.3 (-2.6, 27.1)
Factor VIII (IU/dL)					
Crude	-1 (-4, 1)	1 (-4, 6)	2 (-5, 9)	0 (-11, 11)	18 (8, 28)
Adjusted*	-4 (-6, -1)	-5 (-10, 0)	-3 (-10, 4)	-8 (-18, 1)	12 (3, 22)
Factor IX (IU/dL)†					
Crude	0 (-2, 1)	3 (0, 6)	4 (-1, 9)	2 (-3, 8)	17 (10, 25)
Adjusted*	0 (-2, 1)	1 (-2, 4)	0 (-4, 4)	-2 (-5, 2)	9 (3, 14)
Factor XI (IU/dL)					
Crude	4 (2, 5)	2 (-2, 5)	2 (-2, 7)	3 (-2, 7)	6 (2, 11)
Adjusted*	1 (-1, 2)	-2 (-5, 1)	-2 (-6, 2)	1 (-3, 5)	4 (0, 9)

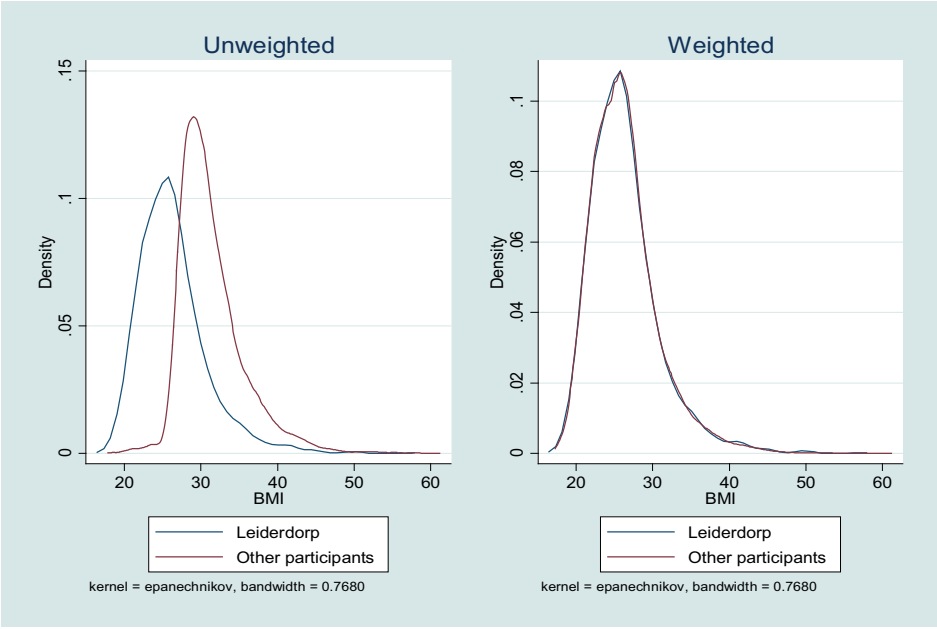
	Crude and adjusted# mean difference (95% CI) compared with <50th percentile (PWV <6.31 m/s)		
oagulation Factor	50th-90th Percentile (PWV ranges, 6.31-8.32 m/s)	90th-95th Percentile (PWV ranges, 8.32-9.14 m/s)	>95th Percentile (PWV >9.14 m/s)
Fibrinogen (g/L)			
Crude	12.5 (5.6, 19.4)	2.7 (-7.7, 13.1)	16.8 (2.4, 31.1)
Adjusted*	1.4 (-4.6, 7.4)	-6.8 (-15.7, 2.1)	11.4 (-2.6, 25.4)
Factor VIII (IU/dL)			
Crude	4 (0, 8)	5 (-2, 12)	5 (-4, 14)
Adjusted*	0 (-4, 4)	1 (-6, 8)	-1 (-9, 8)
Factor IX (IU/dL)†			
Crude	6 (4, 9)	12 (7, 17)	7 (-1, 15)
Adjusted*	3 (1, 5)	9 (5, 13)	7 (-1, 15)
Factor XI (IU/dL)			
Crude	4 (2, 6)	6 (1, 11)	4 (-2, 10)
Adjusted*	3 (0, 5)	3 (-1, 8)	4 (-2, 10)

Results were weighted toward the BMI distribution of the general population (n=6,536). †Vitamin K antagonist users were excluded from the analysis *Adjusted for; sex, age, smoking, total body fat, CRP, total cholesterol, ethnicity and education. Corresponding linear regression coefficients, 95% Confidence Intervals

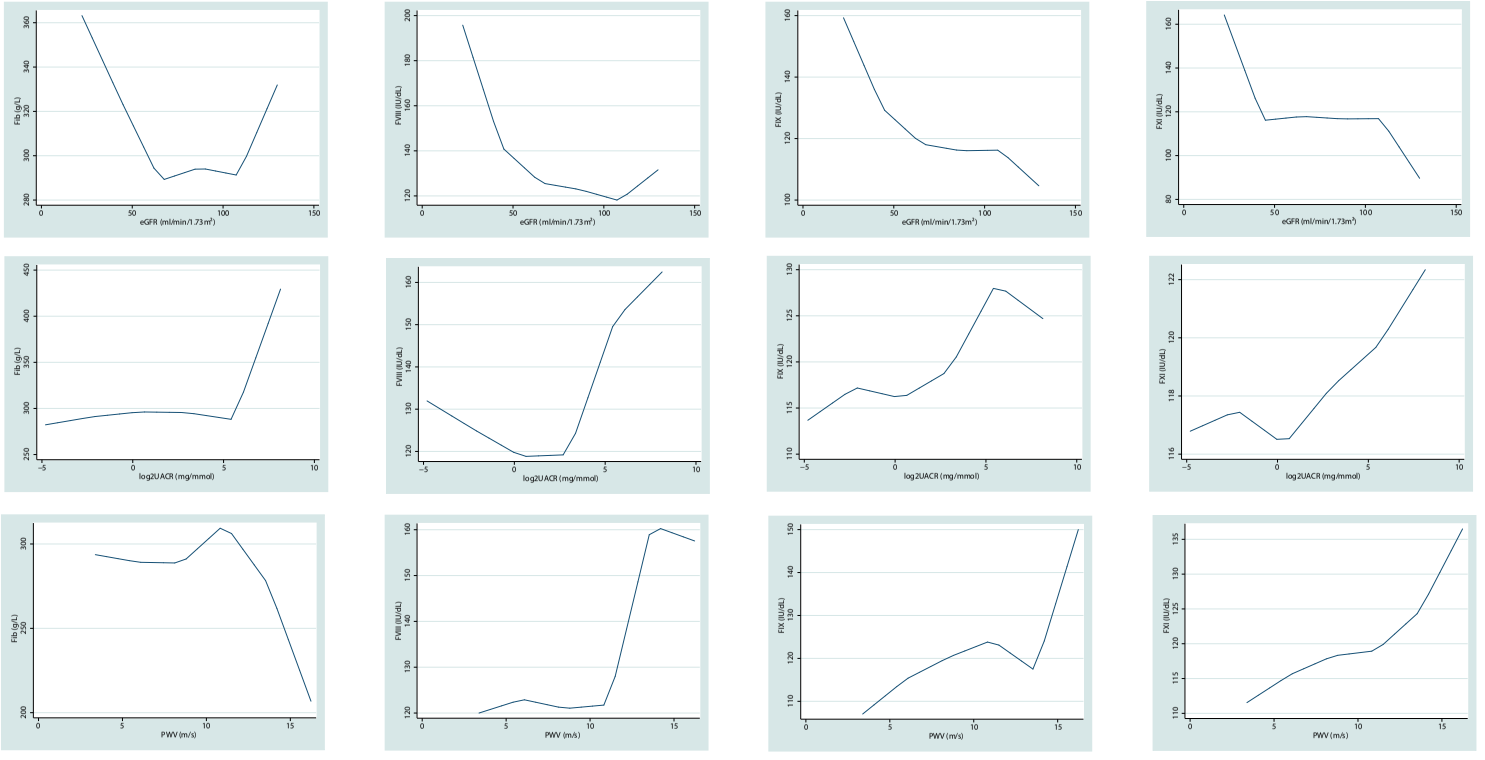
Supplemental Table 3. Weight factors for the different BMI categories of the NEO population, as used in the statistical analyses.

BMI categories (kg/m²)	Weight factor
>=30	1
29-30	1.304461
28-29	1.472934
27-28	2.458912
26-27	4.445434
25-26	8.668198
<25	10.26279

Weight factors were calculated using the BMI distribution of the reference (Leiderdorp) population (n=1,671), whose BMI distribution was similar to the BMI distribution of the general Dutch population (20). All results were based on weighted analyses. Consequently, the results apply to a population-based study without oversampling of individuals with a BMI ≥ 27 kg/m^2.



Supplemental Figure 1. Distribution of BMI in the Leiden population (blue) and in the total NEO population (red) before weighting (left) and after weighting (right). The unweighted and weighted density plots show that weighting corrects for oversampling of participants with a BMI >27 kg/m^2, and that the BMI distribution in the NEO population after weighting is similar to the BMI distribution of the general population.



Supplemental Figure 2. Spline models showing the association between eGFR (upper row), UACR (middle row), PWV (lower row), and coagulation factors fibrinogen, FVIII, FIX, and FXI.