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Complete List of Authors:	Huang, Meng-Jie Wei, Ri-bao Wang, Yang Su, Ting-yu Di, Ping; Department of Clinical Laboratory, Chinese PLA General Hospital, Beijing, China Li, Qing-ping Yang, Xi Li, Ping Chen, Xiang-mei
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Blood coagulation system in patients with chronic kidney disease: a prospective observational study

Meng-Jie Huang[a], Ri-bao Wei[a]*, Yang Wang[a], Ting-yu Su[a], Ping Di[b], Qing-ping Li[a], Xi Yang[a], Ping Li[a], Xiang-mei Chen[a]

[a]Department of Nephrology, Chinese PLA General Hospital, Chinese PLA Institute of Nephrology, State Key Laboratory of Kidney Diseases, National Clinical Research Center for Kidney Diseases, Beijing, China

[b]Department of Clinical Laboratory, Chinese PLA General Hospital, Beijing, China

*Corresponding author: Ri-bao Wei, MD, Department of Nephrology, Chinese PLA General Hospital, Chinese PLA Institute of Nephrology, State Key Laboratory of Kidney Diseases, National Clinical Research Center for Kidney Diseases, Beijing 100853, People's Republic of China

Email: wrbbj2006@126.com Tel: 86-10-55499133 Fax: 86-10-88626068

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Abstract

Objectives: Thromboembolic events are the major factor affecting the prognosis of chronic kidney disease (CKD) patients. Hemostatic alterations are possible causes of these complications, but their roles and profiles remain poorly characterized. In the prospective observational study, we investigated the entire coagulation process in CKD patients to elucidate the mechanisms underlying thrombophilia.

Methods: A total of 95 CKD patients and 20 healthy controls who met the inclusion criteria were consecutively recruited from September 2015 to March 2016. The platelet count, von Willebrand factor antigen (vWF:Ag), vWF ristocetin cofactor activity (vWF:RCo), fibrinogen, factor V (FV), FVII, FVIII, antithrombin III, protein C, protein S, D-dimer, standard coagulation tests, and thromboelastography were measured in the CKD patients and controls. Associations between the estimated glomerular filtration rate (eGFR) and hemostatic biomarkers were tested using multivariable linear regression.

Results: After adjustment for demographics and comorbidities, the vWF:Ag, vWF:RCo, fibrinogen, FVII, FVIII, and D-dimer levels were significantly higher in the CKD patients than in the healthy controls, and were elevated with CKD progression. However, the adjusted thromboelastography parameters showed no significant differences in the R, K, MA, and angle values between the CKD patients and healthy controls. In the correlation analysis, vWF Ag, vWF:RCo, and FVIII were clearly inversely associated with eGFR ($r = -0.359$, $P<0.001$; $r = -0.391$, $P<0.001$; $r = -0.327$, $P<0.001$, respectively).

Conclusions: CKD patients are characterized by endothelial dysfunction and enhanced coagulation, especially FVIII activity. The abnormal hemostatic profiles may contribute to the

elevated risk of thrombotic events.

Strengths and limitations of this study

- Existing studies on the mechanism of coagulation in CKD are mostly limited to hemodialysis patients with end-stage renal disease. The changes in the coagulation function of non-dialysis patients with moderate to severe CKD have not been completely clarified. In our research article, we investigated the entire coagulation process in non-dialysis CKD patients.
- We found that CKD patients are characterized by endothelial dysfunction and enhanced coagulation, especially FVIII activity. Besides, we also performed thromboelastography for dynamic observation of the entire coagulation process in CKD patients but detected no changes in the coagulation function.
- Our study is limited by the limited methods available for platelet function testing at our center. We did not verify platelet function changes in the CKD patients apart from thromboelastography. Also, the present study was a cross-sectional study that did not follow-up the patients or establish a relationship between the elevation of procoagulant factors and eventual subsequent thromboembolic events in the CKD patients.

Introduction

Chronic kidney disease (CKD) patients commonly have blood coagulation disorders. The resulting thrombotic complications have become the most common cause of death and one of the difficulties in renal replacement therapy among CKD patients.[1-4] Existing studies on the mechanism of coagulation in CKD are mostly limited to hemodialysis patients with end-stage renal disease (ESRD).[5-7] The changes in the coagulation function of non-dialysis patients with moderate to severe CKD have not been completely clarified.

The coagulation process involves the participation of the platelets, vascular endothelium, coagulation system, anticoagulant system, and fibrinolytic system. Most coagulation test methods reflect changes in a particular blood coagulation step but have difficulty completely verifying the entire coagulation process in CKD patients. In the present study, several coagulation test methods were used to measure markers of endothelial function [von Willebrand factor antigen (vWF:Ag) and vWF ristocetin cofactor activity (vWF:RCo)], the major blood coagulation pathways [fibrinogen, factor V (FV), FVII, and FVIII], and natural coagulation inhibitors (antithrombin III, protein S and protein C). Additionally, standard coagulation tests and thromboelastography (TEG) were adopted for dynamic observation of the entire coagulation process. The purpose of the study was to investigate the entire coagulation process in non-dialysis patients at different CKD stages to elucidate the mechanisms underlying thrombophilia and guide antithrombotic treatment.

Methods

1. Study design and subjects

This prospective observational study was performed at the Department of Nephrology, Chinese PLA General Hospital. Between September 2015 and March 2016, consecutive patients 18 to 70 years of age with CKD who were not receiving dialysis were included in this study. The exclusion criteria were as follows: (1) patients with secondary renal disease [diabetic nephropathy, lupus nephritis, or antineutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis)]; (2) patients with nephrotic syndrome; (3) patients with signs of acute infection, liver failure, trauma, surgery, cancer, or pregnancy; (4) patients on glucocorticoids, immunosuppressive medication and anticoagulant medication within the past month; and (5) patients with a history of previous thromboembolic or hemorrhagic events. Finally, 95 patients with CKD met the exclusion criteria and agreed to participate in the study. Additionally, 20 age- and gender-matched healthy controls with no history of kidney disease who met the same exclusion criteria were recruited. Informed consent was obtained from all individuals included in this study and the research was approved by the ethics committee of the General Hospital of the Chinese People's Liberation Army. A flowchart is shown in Figure 1.

Figure 1. The flow chart of this study

2. General data collection

We recorded the subjects' general conditions (age, gender, height, weight, systolic blood pressure, diastolic blood pressure, and smoking history), underlying diseases [coronary heart disease (CHD) and diabetes mellitus], and laboratory parameters [hemoglobin, white blood cell count, platelet count, serum albumin, serum creatinine, cholesterol, triglycerides, and urinary albumin to creatinine ratio (UACR)].

We also calculated the body mass index (BMI) and mean arterial pressure (MAP) as follows: BMI = weight (kg)/[height (m)]^2 and MAP = (systolic blood pressure + 2·diastolic blood pressure)/3.

The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to estimate the glomerular filtration rate (eGFR).[8] The CKD stage was defined according to the Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines.[9]

3. Procoagulant and anticoagulant factors

Fasting cubital venous blood specimens were collected in the morning and mixed with 109 mmol/L sodium citrate for anticoagulation (sodium citrate:blood = 1:9). The blood samples were centrifuged at 3000 x g for 10 min within 1 h of collection to obtain platelet-poor plasma. Factor V, VII, and VIII activities as well as the anticoagulant factors protein C and protein S were analyzed by clotting assays. vWF:Ag and vWF:RCo were measured by immunoturbidimetric assay. All instruments (ACL TOP700) and reagents were purchased from USA Instrumentation Laboratory Company.

4. Standard coagulation tests

The activated partial thromboplastin time (APTT), prothrombin time (PT), and fibrinogen were analyzed by the magnetic bead assay. Antithrombin III was analyzed by the chromogenic substrate assay. The D-dimer content was measured by immunoturbidimetry using a device and reagents purchased from Stago (France).

5. Thromboelastography (TEG)

The coagulation status was assessed via TEG using citrated whole blood samples. For each TEG assay, citrated whole blood (1 ml) was pipetted into a vial containing 1% kaolin

and inverted 5 times to ensure mixing of kaolin with the blood. Then, 340 µl of kaolin-activated citrated whole blood was transferred to a TEG cup to which 20 µl of 0.2 mol/l $CaCl^2$ had been preloaded for recalcification. The TEG analyzer was stopped 40-60 minutes after reaching the maximum amplitude at 37°C. The parameters included (1) reaction time (R) - time from the start of the test to a TEG amplitude of 2 mm, reflecting the combined effect of the coagulation factors involved in the initiation of hemostasis; (2) K-time (K) - the period from the TEG amplitude of 2 mm to when the curve reached an amplitude of 20 mm, which measured the rate of clot formation (fibrin cross-linking); (3) α-angle - the angle between the tangent line (drawn from the split point to the curve) and the horizontal base line, representing the acceleration of fibrin build-up and cross-linking; and (4) maximum amplitude (MA) - indicative of the strength of the clot that reflected the cross interaction between platelet functions and coagulation.

6. Statistical analysis

Data analysis was performed using SPSS software, version 19.0 (Chicago, IL, USA). The results are expressed as the mean ± standard deviation or the median (range) for continuous data and as a frequency or percentage for categorical data. We initially compared baseline characteristics among the CKD patients and healthy controls using analysis of variance (ANOVA), Kruskal-Wallis test or Chi-squared test as appropriate. A generalized linear model estimating procedure was used to obtain adjusted mean levels of procoagulant biomarkers within renal function categories. Using multivariable linear regression, we examined the association of eGFR with hemostatic biomarkers. eGFR and other baseline characteristics

were the independent variables and the biomarkers were the dependent variables in these analyses. P values less than 0.05 were considered statistically significant.

Results

Participants' characteristics

Baseline characteristics of the CKD patients and healthy controls are shown in Table 1. No significant differences were detected in age, gender ratio, BMI, white blood cell, or cholesterol between the CKD patients and healthy controls. Subjects with CKD stage 5 (CKD 5) had higher MAP, triglyceride, and UACR but lower hemoglobin and serum albumin levels than the healthy controls. Given the small number of subjects with concomitant CHD, diabetes mellitus, and smoking in the CKD and healthy control groups, we combined CKD stage 3–5 patients for comparison with the healthy controls. However, no significant differences were found between the CKD patients and healthy controls regarding CHD, diabetes mellitus, or smoking ratio.

Table 1. Characteristics of CKD patients and healthy controls

Variables	Healthy control	CKD3	CKD4	CKD5	P
No. of patients	20	32	38	25	
Gender, M, n (%)	9(47%)	22(69%)	25(66%)	13(52%)	0.225
Age (year)	39.7±16.7	40.3±11.3	44.5±14.4	44.0±13.7	0.443
BMI (kg/ m^2)	23.3±4.3	24.4±4.2	24.7±3.6	23.8±4.2	0.582
MAP (mmHg)	89.5±9.3◆	95.0±9.4◆	97.5±8.5*◆	103.8±17.2*	<0.001
Hemoglobin (g/L)	136.3±17.4◆	131.2±20.2◆	118.9±18.1*◆	98.5±14.2*	<0.001

White blood cell (10^9/l)	6.1±1.6	7.1±2.1	7.1±1.5	6.9±2.2	0.398
Serum albumin (g/L)	43.2±2.9◆	41.1±3.4	40.7±3.4	39.1±3.6*	0.004
Serum creatinine (umol/l)	69.4±13.5◆	155.5±31.5*◆	265.7±63.4*◆	542.1±237.1*	<0.001
eGFR (ml/min/1.73 m^2)	101.4±21.9◆	48.9±13.9*◆	24.7±6.3*◆	10.9±4.1*	<0.001
Cholesterol (mmol/l)	4.2±0.9	4.2±1.2	4.2±0.8	4.0±0.9	0.959
Triglyceride (mmol/l)	1.2±0.6	1.9±0.9*	2.3±1.1*◆	1.7±0.8	0.003
UACR (mg/g)	7.3±2.7◆	201.9±142.7*◆	313.8±139.5*◆	472.2±224.1*	<0.001
CHD, n (%)	2 (10%)	1 (3%)	3 (8%)	1 (4%)	0.753
Diabetes Mellitus, n (%)	1(5%)	2 (6%)	3 (8%)	3 (11%)	0.523
Current smoking,n (%)	2(10%)	7 (21%)	6 (15%)	1 (4%)	0.457

Data are expressed as mean±standard deviation (SD) or median (interquatile range) as appropriate ; BMI=body mass index; MAP=mean arterial pressure; eGFR=estimated glomerular filtration rate; UACR=Urine Albumin/Creatinine ratio; CHD=coronary artery disease.

*p<0.05, vs control group;

◆p<0.05, vs CKD 5 group.

Procoagulant biomarkers according to chronic kidney disease

Table 2 shows the unadjusted and adjusted means of the procoagulant biomarkers by CKD status. The FVII, FVIII, vWF:Ag, vWF:RCo, fibrinogen, and D-dimer levels were significantly higher in the CKD patients than in the controls after adjustment for age, gender, history of diabetes, history of CHD, smoking status, MAP, BMI, hemoglobin, serum albumin, cholesterol, triglyceride, and UACR. With the exception of FVII, all of these parameters were elevated with CKD progression. Prior to adjustment, protein C was significantly lower in the CKD 5 patients than in the controls; however, after adjustment, no significant difference was found in this parameter between groups (P=0.736). Irrespective of the adjustment, platelet count, FV, antithrombin III, APTT, and PT showed no significant differences between the CKD 3–5 patients and the healthy controls.

Table 2. Unadjusted and adjusted levels of procoagulant biomarkers.

Variables	Unadjusted					Multivariable-adjusted*				
	Healthy control	CKD3	CKD4	CKD5	P	Healthy control	CKD3	CKD4	CKD5	P
Platelet (10^9/l)	237.2±47.4	214.8±65.0	214.0±52.8	195.8±58.6	0.156	239.3±72.4	218.1±60.5	211.4±57.9	186.3±71.9	0.284

Factor V (%)	113.6±26.1	98.4±31.9	106.7±36.9	103.4±33.3	0.533	108.3±38.5	99.7±35.0	108.0±32.6	99.6±40.3	0.640
Factor VII(%)	74.2±14.3◆	94.5±18.0*◆	104.2±17.9*	108.4±27.2*	<0.001	78.8±25.2◆	99.4±23.4*	104.0±20.9*◆	97.7±27.4*	0.050
Factor VIII(%)	86.5±22.3◆	115.3±25.1*◆	130.5±27.6*	139.9±33.0*	<0.001	82.3±31.6◆	118.2±28.9*◆	129.7±27.1*	141.1±32.8*	<0.001
VWF:Ag(%)	103.1±42.4◆	124.7±51.4◆	158.9±49.9*	181.8±45.6*	<0.001	92.9±51.4◆	131.0±51.1*◆	155.8±46.2*	182.9±56.4*	0.011
vWF:RCo(%)	99.8±29.9◆	115.5±43.2◆	150.2±45.1*	168.2±41.5*	<0.001	86.4±44.1◆	120.9±44.1*◆	147.9±40.0*	170.0±48.8*	0.004
Fibrinogen(g/l)	3.0±0.8◆	3.1±0.7◆	3.8±0.8*◆	4.5±1.1*	<0.001	3.7±1.0◆	3.2±0.9*◆	3.7±0.9◆	4.2±1.0*	0.006
Protein C(%)	105.3±17.0◆	99.4±18.6◆	93.5±17.9	86.6±15.2*	0.024	98.2±19.2	97.1±16.6	92.5±15.5	93.3±19.6	0.736
Protein S(%)	76.8±23.2	88.2±24.6	94.5±20.7	99.5±25.5	0.076	83.7±29.1	87.1±25.8	93.4±23.5	99.4±30.0	0.584
AT III (%)	99.5±9.3	103.8±12.2	103.8±11.7	103.1±11.8	0.658	99.2±12.9	104.7±11.3	104.2±10.4	99.2±12.9	0.189
D-dimer,(ng/ml)	257±116◆	425±277◆	505±320*◆	842±496*	<0.001	362±404◆	464±367◆	505±345*◆	780±422*	0.039
APTT(s)	39.0±4.5	37.7±3.2	37.5±3.7	39.0±4.2	0.286	40.5±4.8	37.8±3.9	37.5±3.6	38.2±4.8	0.187
PT(s)	13.4±0.6	13.5±0.6	13.5±0.6	13.7±0.6	0.320	137±0.7	13.5±0.5	13.5±0.6	13.8±0.7	0.192

Data are adjusted for age, sex, history of diabetes, history of CHD, smoking status, MAP, BMI, hemoglobin, serum albumin, cholesterol, triglyceride, and UACR.

AT III=antithrombin III; APTT=activated partial thromboplastin time; PT=prothrombin time.

*p<0.05, vs control group; ◆p<0.05, vs CKD 5 group.

Thromboelastography

Figure 2 compares the unadjusted and adjusted TEG parameters between the CKD patients and the healthy controls. The results showed that the R time and K time in the unadjusted cohort were hypercoagulable in the CKD 4–5 patients compared with the CKD 3 patients and the healthy controls ($p < 0.05$). The MA values in the CKD 5 group were significantly higher than the values in the control group (63.3±9.3 mm versus 57.9±5.7 mm, p=0.046). However, after adjustment for relevant factors, no significant differences were found in the R, K, MA, and a-angle values between the CKD patients and the healthy controls.

Figure 2. Unadjusted and adjusted TEG parameters in healthy controls, CKD3-5 stage.

Data are adjusted for age, sex, history of diabetes, history of CHD, smoking status, MAP, BMI, hemoglobin, serum albumin, cholesterol, triglyceride, and UACR.

(A) Unadjusted and Adjusted R value. (B) Unadjusted and Adjusted K value. (C) Unadjusted and Adjusted MA value. (D) Unadjusted and Adjusted α-angle value.

*p<0.05, vs control group; ◆p<0.05, vs CKD 5 group.

Associations between renal function and hemostatic biomarkers

As shown in Figure 3, vWF Ag, vWF:RCo, FVIII were inversely correlated with eGFR (r = −0.359, P=0.001; r = −0.391, P<0.001; r = −0.327, P=0.001). Besides, we also used multivariable linear regression to analyze the associations between eGFR and hemostatic biomarkers. Table 3 presents multivariate-adjusted regression

coefficients (95% confidence intervals). In the multivariate-adjusted models, higher vWF Ag, vWF:RCo, FVIII were significantly associated with a decreased eGFR.

Figure 3. Correlation of vWF Ag, vWF:RCo, and FVIII levels with eGFR.

(A) Correlation of vWF Ag with eGFR. (B) Correlation of vWF:RCo with eGFR. (C) Correlation of FVIII with eGFR.

Table 3. Multivariable-Adjusted Regression Coefficients (95% Confidence Intervals) of hemostatic biomarkers with eGFR.

	eGFR, mL/min/1.73 m 2	
	Effect size (95% CI)	p-value
Factor VIII	-0.50 (-0.69, -0.31)	<0.001
VWF:Ag	-0.92(-1.33, -0.40)	<0.001
vWF:RCo	-0.82 (-1.19, -0.45)	<0.001

*Adjusted for age, sex, history of diabetes, history of CHD, smoking status, MAP, BMI, hemoglobin, serum albumin, eGFR, cholesterol, triglyceride, and UACR.

Discussion

We evaluated the coagulation profiles in CKD patients who were not receiving dialysis using multiple laboratory methods, including the vascular endothelium, coagulation factor, anticoagulation system, conventional blood test, standard coagulation tests, and TEG.

VWF, which is a large molecular weight glycoprotein synthesized and secreted by endothelial cells and megakaryocytes, exerts a procoagulant effect through platelet

adhesion and aggregation and FVIII stabilization.[10] The increased vWF is a sign of endothelial injury and a risk for thromboembolic events.[11, 12] Fibrinogen, FVII, and FVIII are important coagulation factors in the coagulation pathway, whereas D-dimer reflects the activation of the coagulation system and the formation of blood clots in the body. Fibrinogen, FVII, FVIII, and D-dimer have also been shown to be associated with an increased prevalence of thromboembolic events.[13-16]

In our study, we observed elevated D-dimer, fibrinogen, factor VII, and especially factor VIII and vWF levels in CKD patients. And the coagulation was enhanced with the aggravation of renal injury. CKD patients often present higher levels of traditional risk factors for thromboembolic events, such as hypertension, diabetes, obesity and dyslipidemia;[17] these factors also affect the coagulation system. In our attempt to explain hemostatic alterations in chronic kidney disease, we adjusted for the above influencing factors. The results showed that procoagulant factors were still significantly elevated in the CKD patients, indicating that kidney dysfunction affected the activation of coagulation function in addition to traditional risk factors.

Possible mechanisms to explain the association of lower eGFR and higher levels of hemostatic factors are as follows. (1) With CKD progression, renal impairment is aggravated and a large number of renal units are damaged, resulting in the loss of normal excretory function and a reduction in the removal of procoagulant substances. A few studies found that the metabolism and elimination of fibrinogen and D-dimer were decreased in CKD and ESRD.[18-20] (2) The increase in the FVII level may be due to vascular endothelial damage in CKD patients, resulting in tissue factor

expression.[21] (3) Moreover, extensive research has found that vWF, fibrinogen, and FVIII are associated with the inflammatory response.[22] CKD patients are commonly associated with changes in the levels of various inflammatory cytokines.[23] Proinflammatory substances can activate procoagulant factors and result in elevated levels of particular hemostatic factors.

TEG displays blood clot formation dynamics from initial thrombin generation to fibrinolysis.[24] In the current study, we also performed TEG for dynamic observation of the entire coagulation process in CKD patients. Prior to adjustment for confounding factors, the TEG data suggested that all aspects of coagulation were increased in the CKD patients, including initial fibrin formation, fibrin-platelet interactions, and qualitative platelet functions. However, after adjustment for relevant influencing factors, we found no significant differences in the TEG parameters (R, K, MA, and angle) between the CKD patients and the healthy controls, which is in contrast to previous TEG studies in hemodialysis patients with ESRD.[25, 26] Hemodialysis patients are influenced by hemodynamic factors and coagulant use and thus present more complicated changes in coagulation functions, which are different from those in non-dialysis CKD patients.[5] Thus, whether TEG can be used to effectively evaluate the integrated coagulation function in non-dialysis CKD patients requires further validation.

The present study has certain limitations. First, platelet dysfunction was previously thought to play a role in coagulation disorders among CKD patients. However, due to the limited methods available for platelet function testing at our

center, we did not verify platelet function changes in the CKD patients or thoroughly explore the role of platelets apart from TEG. Second, the present study was a cross-sectional study that did not follow-up the patients or establish a relationship between the elevation of procoagulant factors and eventual subsequent thromboembolic events in the CKD patients.

Conclusions

In conclusion, CKD patients are characterized by endothelial dysfunction and enhanced coagulation, especially FVIII activity. The abnormal hemostatic profiles may contribute to the elevated risk of thrombotic events. TEG detected no changes in the coagulation function among the CKD patients. Whether TEG can effectively evaluate the integrated coagulation function in CKD patients needs to be verified using larger samples. Future studies are required to target the role of coagulation management for CKD patients to reduce co-morbidities.

Contributors: H-MJ. W-RB and C-XM created and designed this study. H-MJ. W Y. S-TY. L-QP and Y X collected and analysed the data. H-MJ. W-RB. D P and L P contributed to the preparation and editing of the manuscript.

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Data Sharing: No Data available

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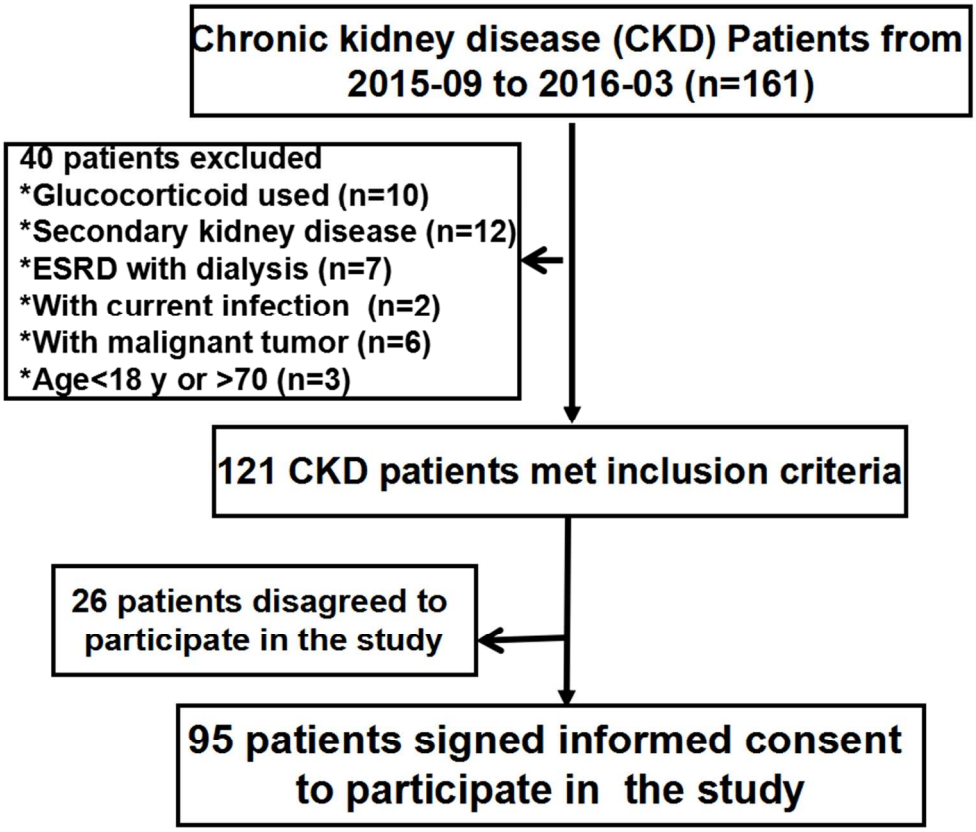

Figure 1. The flow chart of this study

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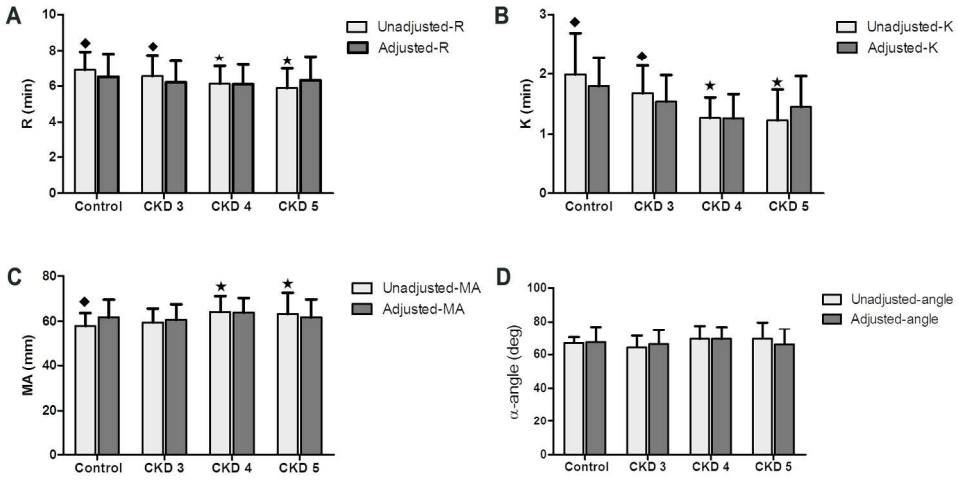

Figure 2. Unadjusted and adjusted TEG parameters in healthy controls, CKD3-5 stage.
Data are adjusted for age, sex, history of diabetes, history of CHD, smoking status, MAP, BMI, hemoglobin, serum albumin, cholesterol, triglyceride, and UACR.
(A) Unadjusted and Adjusted R value. (B) Unadjusted and Adjusted K value. (C) Unadjusted and Adjusted MA value. (D) Unadjusted and Adjusted α-angle value.
*p<0.05, vs control group; ◆p<0.05, vs CKD 5 group.

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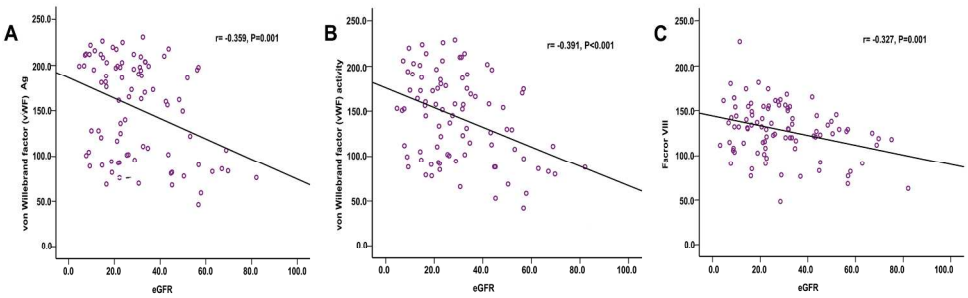

Figure 3. Correlation of vWF Ag, vWF:RCo, and FVIII levels with eGFR.
(A) Correlation of vWF Ag with eGFR. (B) Correlation of vWF:RCo with eGFR. (C) Correlation of FVIII with eGFR.

230x80mm (300 x 300 DPI)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 4
Objectives	3	State specific objectives, including any pre-specified hypotheses	Page 4
Methods			
Study design	4	Present key elements of study design early in the paper	Page 5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 5-7
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Page 5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 5-7
Bias	9	Describe any efforts to address potential sources of bias	Page 8
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 8
		(*b*) Describe any methods used to examine subgroups and interactions	Page 8
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	Page 8

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Page 8,9
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	Page 6
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 8,9
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 11-15
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 16
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 16-18
Generalisability	21	Discuss the generalisability (external validity) of the study results	
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 19

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Blood coagulation system in patients with chronic kidney disease: a prospective observational study

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Blood coagulation system in patients with chronic kidney disease: a prospective observational study

Meng-Jie Huang[a], Ri-bao Wei[a]*, Yang Wang[a], Ting-yu Su[a], Ping Di[b], Qing-ping Li[a], Xi Yang[a], Ping Li[a], Xiang-mei Chen[a]

[a]Department of Nephrology, Chinese PLA General Hospital, Chinese PLA Institute of Nephrology, State Key Laboratory of Kidney Diseases, National Clinical Research Center for Kidney Diseases, Beijing, China

[b]Department of Clinical Laboratory, Chinese PLA General Hospital, Beijing, China

*Corresponding author: Ri-bao Wei, MD, Department of Nephrology, Chinese PLA General Hospital, Chinese PLA Institute of Nephrology, State Key Laboratory of Kidney Diseases, National Clinical Research Center for Kidney Diseases, Beijing 100853, People's Republic of China

Email: wrbbj2006@126.com Tel: 86-10-55499133 Fax: 86-10-88626068

Number of Words: 3130

Abstract

Objectives: Thromboembolic events are the major factor affecting the prognosis of chronic kidney disease (CKD) patients. Hemostatic alterations are possible causes of these complications, but their roles remain poorly characterized. In the prospective observational study, we investigated the entire coagulation process in CKD patients to elucidate the mechanisms of their high thromboembolic risk.

Methods: A total of 95 CKD patients and 20 healthy controls who met the inclusion criteria were consecutively recruited from September 2015 to March 2016. The platelet count, platelet aggregation, von Willebrand factor antigen (vWF:Ag), vWF ristocetin cofactor activity (vWF:RCo), fibrinogen, factor V (FV), FVII, FVIII, antithrombin III, protein C, protein S, D-dimer, standard coagulation tests, and thromboelastography were measured in the CKD patients and controls. Associations between the estimated glomerular filtration rate (eGFR) and hemostatic biomarkers were tested using multivariable linear regression.

Results: The adjusted and unadjusted levels of vWF:Ag, vWF:RCo, fibrinogen, FVII, FVIII, and D-dimer were significantly higher in the CKD patients than that in the healthy controls, and were elevated with CKD progression. However, after adjustment for baseline differences, platelet aggregation and thromboelastography parameters showed no significant differences between the CKD patients and healthy controls. In the correlation analysis, vWF Ag, vWF:RCo, and FVIII were inversely associated with eGFR ($r = -0.359$, $P<0.001$; $r = -0.391$, $P<0.001$; $r = -0.327$, $P<0.001$, respectively). During the one year of follow up, one cardiovascular event

occurred in patients with CKD 5 stage, whereas none thromboembolic event occurred in the CKD 3-4 and control groups.

Conclusions: CKD patients are characterized by endothelial dysfunction and increased coagulation, especially FVIII activity. The abnormal hemostatic profiles may contribute to the elevated risk of thrombotic events but further longer-term study with large samples are still required to more precisely determine the relationship between the elevation of procoagulant factors and clinical outcomes.

Strengths and limitations of this study

- Existing studies on the mechanism of coagulation in CKD are mostly limited to patients with end-stage renal disease requiring hemodialysis. The changes in the coagulation function of non-dialysis patients with moderate to severe CKD have not been completely clarified. In our research article, we investigated the entire coagulation process in non-dialysis CKD patients.
- We found that CKD patients are characterized by endothelial dysfunction and increased coagulation, especially FVIII activity. Besides, we also performed thromboelastography for dynamic observation of the entire coagulation process in CKD patients but detected no changes in the coagulation function.
- Due to the limited sample size and short term of follow-up, we might underestimate the risk of thromboembolic events in CKD patients, which were not well linked the observations with clinical outcomes.

Introduction

Chronic kidney disease (CKD) patients commonly have blood coagulation disorders. The resulting thrombotic complications have become the most common cause of death and one of the difficulties in renal replacement therapy among CKD patients.[1-4] Existing studies on the mechanism of coagulation in CKD are mostly limited to hemodialysis patients with end-stage renal disease (ESRD).[5-7] The changes in the coagulation function of non-dialysis patients with moderate to severe CKD have not been completely clarified.

The coagulation process involves the participation of the platelets, vascular endothelium, coagulation system, anticoagulant system, and fibrinolytic system. Most coagulation test methods reflect changes in a particular blood coagulation step but have difficulty completely verifying the entire coagulation process in CKD patients. In the present study, several coagulation test methods were used to measure markers of platelet [platelet counts, platelet aggregability], endothelial function [von Willebrand factor antigen (vWF:Ag) and vWF ristocetin cofactor activity (vWF:RCo)], the major blood coagulation pathways [fibrinogen, factor V (FV), FVII, and FVIII], and natural coagulation inhibitors (antithrombin III, protein S and protein C). Additionally, standard coagulation tests and thromboelastography (TEG) were adopted for dynamic observation of the entire coagulation process. The purpose of the study was to investigate the entire coagulation process in non-dialysis patients at different CKD stages to elucidate the mechanisms of their high thromboembolic risk and guide antithrombotic treatment.

Methods

1. Study design and subjects

This prospective observational study was performed at the Department of Nephrology, Chinese PLA General Hospital. Between September 2015 and March 2016, consecutive patients 18 to 70 years of age with stages 3–5 non-dialysis-dependent CKD were included in this study. The exclusion criteria were as follows: (1) patients with secondary renal disease [diabetic nephropathy, lupus nephritis, or antineutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis)]; (2) patients with nephrotic syndrome; (3) patients with signs of acute infection, liver failure, trauma, surgery, cancer, or pregnancy; (4) patients on glucocorticoids, immunosuppressive medication and anticoagulant medication within the past 1 month; and (5) patients with a history of previous thromboembolic or hemorrhagic events within 12 months. Finally, 95 patients with CKD met the exclusion criteria and agreed to participate in the study. Additionally, 20 age- and gender-matched healthy controls with no history of kidney disease who met the same exclusion criteria were recruited. Informed consent was obtained from all individuals included in this study and the research was approved by the ethics committee of the General Hospital of the Chinese People's Liberation Army. A flowchart is shown in Figure 1.

Figure 1. The flow chart of this study

2. General data collection

We recorded the subjects' general conditions (age, gender, height, weight, systolic blood pressure, diastolic blood pressure, and smoking history), underlying diseases

[coronary heart disease (CHD) and diabetes mellitus], and laboratory parameters [hemoglobin, white blood cell count, platelet count, serum albumin, serum creatinine, cholesterol, triglycerides, and urinary albumin to creatinine ratio (UACR)].

We also calculated the body mass index (BMI) and mean arterial pressure (MAP) as follows: BMI = weight (kg)/[height (m)]^2 and MAP = (systolic blood pressure + 2·diastolic blood pressure)/3.

The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to estimate the glomerular filtration rate (eGFR).[8] The CKD stage was defined according to the Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines.[9]

3. Procoagulant and anticoagulant factors

Fasting cubital venous blood specimens were collected in the morning and mixed with 109 mmol/L sodium citrate for anticoagulation (sodium citrate:blood = 1:9). The blood samples were centrifuged at 3000rpm for 10 min within 1 h of collection to obtain platelet-poor plasma. Factor V, VII, and VIII activities as well as the anticoagulant factors protein C and protein S were analyzed by clotting assays. vWF:Ag and vWF:RCo were measured by immunoturbidimetric assay. All instruments (ACL TOP700) and reagents were purchased from USA Instrumentation Laboratory Company.

4. Platelet aggregation tests

Platelet aggregation was measured by light transmittance aggregometry (LTA). Citrate-anticoagulated whole blood was centrifuged at 800rpm for 5 minutes to obtain

platelet-rich plasma. Platelet-poor plasma was obtained from the remaining specimen by further centrifugation at 3000rpm for 10 minutes. Platelet-rich plasma was adjusted to reach a platelet count of 250×10^9/L. Platelet aggregability was assessed at 37℃ with an AggRam aggregometer (Helena Laboratories, Corp., Beaumont, TX, USA). Platelets were stimulated by 10 μmol/L adenosine diphosphate (ADP). Aggregation was expressed as the maximum percent change in light transmittance from baseline, with platelet-poor plasma as a reference.

5. Standard coagulation tests

The activated partial thromboplastin time (APTT), prothrombin time (PT), and fibrinogen were analyzed by the magnetic bead assay. Antithrombin III was analyzed by the chromogenic substrate assay. The D-dimer content was measured by immunoturbidimetry using a device and reagents purchased from Stago (France).

6. Thromboelastography (TEG)

The coagulation status was assessed via TEG using citrated whole blood samples. For each TEG assay, citrated whole blood (1 ml) was pipetted into a vial containing 1% kaolin and inverted 5 times to ensure mixing of kaolin with the blood. Then, 340 μl of kaolin-activated citrated whole blood was transferred to a TEG cup to which 20 μl of 0.2 mol/l $CaCl^2$ had been preloaded for recalcification. The TEG analyzer was stopped 40-60 minutes after reaching the maximum amplitude at 37°C. The parameters included (1) reaction time (R) - time from the start of the test to a TEG amplitude of 2 mm, reflecting the combined effect of the coagulation factors involved in the initiation of hemostasis; (2) K-time (K) - the period from the TEG amplitude of

2 mm to when the curve reached an amplitude of 20 mm, which measured the rate of clot formation (fibrin cross-linking); (3) α-angle - the angle between the tangent line (drawn from the split point to the curve) and the horizontal base line, representing the acceleration of fibrin build-up and cross-linking; and (4) maximum amplitude (MA) - indicative of the strength of the clot that reflected the cross interaction between platelet functions and coagulation.

7. Thromboembolic events

The incidence of thromboembolic events in the CKD patients and healthy controls were recorded during the one-year of follow-up. Evidence suggests that patients with suspected thromboembolic events should be managed with a diagnostic strategy that includes clinical pre-test probability in the form of prediction scores, D-dimer test, and appropriate clinical imaging results.[10]

6. Statistical analysis

Data analysis was performed using SPSS software, version 19.0 (Chicago, IL, USA). The results are expressed as the mean ± standard deviation or the median (range) for continuous data and as a frequency or percentage for categorical data. We initially compared baseline characteristics among the CKD patients and healthy controls using analysis of variance (ANOVA), Kruskal-Wallis test or Chi-squared test as appropriate. A generalized linear model estimating procedure was used to obtain adjusted mean levels of procoagulant biomarkers within renal function categories. Using multivariable linear regression, we examined the association of eGFR with hemostatic biomarkers. eGFR and other baseline characteristics were the independent

variables and the biomarkers were the dependent variables in these analyses. P values less than 0.05 were considered statistically significant.

Results

Participants' characteristics

Baseline characteristics of the CKD patients and healthy controls are shown in Table 1. No significant differences were detected in age, gender ratio, BMI, white blood cell, or cholesterol between the CKD patients and healthy controls. Subjects with CKD stage 5 (CKD 5) had higher MAP, triglyceride, and UACR but lower hemoglobin and serum albumin levels than the healthy controls. Given the small number of subjects with concomitant CHD, diabetes mellitus, and smoking in the CKD and healthy control groups, we combined CKD stage 3–5 patients for comparison with the healthy controls. However, no significant differences were found between the CKD patients and healthy controls regarding CHD, diabetes mellitus, or smoking ratio.

Table 1. Characteristics of chronic kidney disease patients and healthy controls

Variables	Healthy control	CKD3	CKD4	CKD5	P
No. of patients	20	32	38	25	
Gender, M, n (%)	9(47%)	22(69%)	25(66%)	13(52%)	0.225
Age (year)	39.7±16.7	40.3±11.3	44.5±14.4	44.0±13.7	0.443
BMI (kg/ m^2)	23.3±4.3	24.4±4.2	24.7±3.6	23.8±4.2	0.582
MAP (mmHg)	89.5±9.3◆	95.0±9.4◆	97.5±8.5*◆	103.8±17.2*	<0.001

Hemoglobin (g/L)	136.3±17.4◆	131.2±20.2◆	118.9±18.1*◆	98.5±14.2*	<0.001
White blood cell (10^9/l)	6.1±1.6	7.1±2.1	7.1±1.5	6.9±2.2	0.398
Serum albumin (g/L)	43.2±2.9◆	41.1±3.4	40.7±3.4	39.1±3.6*	0.004
Serum creatinine (umol/l)	69.4±13.5◆	155.5±31.5*◆	265.7±63.4*◆	542.1±237.1*	<0.001
eGFR (ml/min/1.73 m^2)	101.4±21.9◆	48.9±13.9*◆	24.7±6.3*◆	10.9±4.1*	<0.001
Cholesterol (mmol/l)	4.2±0.9	4.2±1.2	4.2±0.8	4.0±0.9	0.959
Triglyceride (mmol/l)	1.2±0.6	1.9±0.9*	2.3±1.1*◆	1.7±0.8	0.003
UACR (mg/g)	7.3±2.7◆	201.9±142.7*◆	313.8±139.5*◆	472.2±224.1*	<0.001
CHD, n (%)	2 (10%)	1 (3%)	3 (8%)	1 (4%)	0.753
Diabetes Mellitus, n (%)	1(5%)	2 (6%)	3 (8%)	3 (11%)	0.523
Current smoking,n (%)	2(10%)	7 (21%)	6 (15%)	1 (4%)	0.457

Data are expressed as mean±standard deviation (SD) or median (interquatile range) as appropriate; BMI=body mass index; MAP=mean arterial pressure; eGFR=estimated glomerular filtration rate; UACR=Urine Albumin/Creatinine ratio; CHD=coronary artery disease.

*p<0.05, vs control group;

◆p<0.05, vs CKD 5 group

Procoagulant biomarkers according to chronic kidney disease

Table 2 shows the procoagulant biomarkers by CKD status. Levels of FVII, FVIII, vWF:Ag, vWF:RCo, fibrinogen, and D-dimer were significantly higher in the CKD patients than the levels in the controls before and after adjustment for age, gender, history of diabetes, history of CHD, smoking status, MAP, BMI, hemoglobin, serum

albumin, cholesterol, triglyceride, and UACR (adjusted values shown in Supplementary Table S1). The magnitude of elevation of the given parameters was proportional to severity of CKD. Prior to adjustment, platelet aggregability was significantly higher and protein C was lower in the CKD 5 patients than that in the controls; however, no significant differences were found after adjustment (P=0.736; P=0.267 respectively). Irrespective of the adjustment, platelet count, FV, antithrombin III, APTT, and PT showed no significant differences among the CKD 3–5 patients and the healthy controls.

In order to make our research more accurate, we further excluded smokers as well as patients with diabetes mellitus and uncontrolled hypertension, and then compared the hemostatic profiles among these patients by CKD status. The results showed that the positive associations of renal insufficiency with these procoagulant biomarkers were similar in participants with or without the above-mentioned comorbidities (Supplementary Table S2).

Table 2. Procoagulant biomarkers by chronic kidney disease status.

Variables	Healthy control	CKD3	CKD4	CKD5	P	#P
Platelet (10^9/l)	237.2±47.4	214.8±65.0	214.0±52.8	195.8±58.6	0.156	0.284
ADP $_{LTA}$ (%)&	64.6±4.8◆	67.3±8.6◆	70.1±8.6	74.7±8.2*	0.041	0.738
Factor V (%)	113.6±26.1	98.4±31.9	106.7±36.9	103.4±33.3	0.533	0.640
Factor VII(%)	74.2±14.3◆	94.5±18.0*◆	104.2±17.9*	108.4±27.2*	<0.001	0.050
Factor VIII(%)	86.5±22.3◆	115.3±25.1*◆	130.5±27.6*	139.9±33.0*	<0.001	<0.001
VWF:Ag(%)	103.1±42.4◆	124.7±51.4◆	158.9±49.9*	181.8±45.6*	<0.001	0.011

vWF:RCo(%)	99.8±29.9◆	115.5±43.2◆	150.2±45.1*	168.2±41.5*	<0.001	0.004
Fibrinogen(g/l)	3.0±0.8◆	3.1±0.7◆	3.8±0.8*◆	4.5±1.1*	<0.001	0.006
Protein C(%)	105.3±17.0◆	99.4±18.6◆	93.5±17.9	86.6±15.2*	0.024	0.736
Protein S(%)	76.8±23.2	88.2±24.6	94.5±20.7	99.5±25.5	0.076	0.584
AT III (%)	99.5±9.3	103.8±12.2	103.8±11.7	103.1±11.8	0.658	0.189
D-dimer (ng/ml)	257±116◆	425±277◆	505±320*◆	842±496*	<0.001	0.039
APTT(s)	39.0±4.5	37.7±3.2	37.5±3.7	39.0±4.2	0.286	0.187
PT(s)	13.4±0.6	13.5±0.6	13.5±0.6	13.7±0.6	0.320	0.192

[#]P-values for the adjusted model. Data are adjusted for age, sex, history of diabetes, history of CHD, smoking status, MAP, BMI, hemoglobin, serum albumin, cholesterol, triglyceride, and UACR.

ADP $_{LTA}$ (%)[&]: Platelet aggregation records were available in 49 CKD cases (15 cases in CKD 3 stage; 21 cases in CKD 4 stage; 13 cases in CKD 5 stage) and 9 healthy controls.

AT III=antithrombin III; APTT=activated partial thromboplastin time; LTA=Light transmittance aggregometry; PT= prothrombin time.

*p<0.05, vs control group; ◆p<0.05, vs CKD 5 group

Thromboelastography

Figure 2 compares the TEG parameters between the CKD patients and the healthy controls. The results showed that the R time and K time in the unadjusted cohort were hypercoagulable in the CKD 4–5 patients compared with the CKD 3 patients and the healthy controls ($p < 0.05$). The MA values in the CKD 5 group were

significantly higher than the values in the control group (63.3±9.3 mm versus 57.9±5.7 mm, p=0.046). However, after adjustment for relevant factors, no significant differences were found in the R, K, MA, and a-angle values between the CKD patients and the healthy controls.

Figure 2. TEG parameters in healthy controls and chronic kidney disease patients.

(A) R value. (B) K value. (C) MA value. (D) α-angle value.

#P-values for the adjusted model. Data are adjusted for age, sex, history of diabetes, history of CHD, smoking status, MAP, BMI, hemoglobin, serum albumin, cholesterol, triglyceride, and UACR.

*p<0.05, vs control group for unadjusted values; ◆p<0.05, vs CKD 5 group for unadjusted values.

Associations between renal function and hemostatic biomarkers

As shown in Figure 3, vWF Ag, vWF:RCo, FVIII were inversely correlated with eGFR (r = −0.359, P=0.001; r = −0.391, P< 0.001; r = −0.327, P=0.001). Besides, we also used multivariable linear regression to analyze the associations between eGFR and hemostatic biomarkers. Adjustment for age, sex, history of diabetes, history of CHD, smoking status, MAP, BMI, hemoglobin, serum albumin, eGFR, cholesterol, triglyceride, and UACR, higher vWF Ag, vWF:RCo and FVIII were significantly associated with a decreased eGFR [Regression Coefficients: -0.92(-1.33, -0.40); -0.82 (-1.19, -0.45); -0.50 (-0.69, -0.31) respectively].

Figure 3. Correlation of vWF Ag, vWF:RCo, and FVIII levels with eGFR.

(A) Correlation of vWF Ag with eGFR. (B) Correlation of vWF:RCo with eGFR. (C) Correlation of FVIII with eGFR. Regression lines: (A) vWF Ag=186.3–1.12×eGFR. (B) vWF:RCo=174.2−1.05×eGFR. (C) FVIII=143.2-0.52×eGFR.

Thromboembolic events

One cardiovascular event (acute myocardial syndrome) occurred in patients with CKD 5 stage, whereas none thromboembolic event occurred in the CKD 3-4 and control groups during the one-year of follow-up.

Discussion

We evaluated the coagulation profiles in CKD patients who were not receiving dialysis using multiple laboratory methods, including the vascular endothelium, coagulation factor, anticoagulation system, conventional blood test, standard coagulation tests, and TEG.

VWF, which is a large molecular weight glycoprotein synthesized and secreted by endothelial cells and megakaryocytes, exerts a procoagulant effect through platelet adhesion and aggregation and FVIII stabilization.[11] The increased vWF is a sign of endothelial injury and a risk for thromboembolic events.[12, 13] Fibrinogen, FVII, and FVIII are important coagulation factors in the coagulation pathway, whereas D-dimer reflects the activation of the coagulation system and the formation of blood clots in the body. Fibrinogen, FVII, FVIII, and D-dimer have also been shown to be associated with an increased prevalence of thromboembolic events.[14-17]

In our study, we observed elevated D-dimer, fibrinogen, factor VII, and especially factor VIII and vWF levels in CKD patients. And the coagulation was increased with

the aggravation of renal injury. CKD patients often present higher levels of traditional risk factors for thromboembolic events, such as hypertension, diabetes, obesity and dyslipidemia;[18] these factors also affect the coagulation system. In our attempt to explain hemostatic alterations in chronic kidney disease, we adjusted for the above influencing factors. The results showed that procoagulant factors were still significantly elevated in the CKD patients, indicating that kidney dysfunction affected the activation of coagulation function in addition to traditional risk factors.

Possible mechanisms to explain the association of lower eGFR and higher levels of hemostatic factors are as follows. (1) With CKD progression, renal impairment is aggravated and a large number of renal units are damaged, resulting in the loss of normal excretory function and a reduction in the removal of procoagulant substances. A few studies found that the metabolism and elimination of fibrinogen and D-dimer were decreased in CKD and ESRD.[19-21] (2) The increase in the FVII level may be due to vascular endothelial damage in CKD patients, resulting in tissue factor expression.[22] (3) Moreover, extensive research has found that vWF, fibrinogen, and FVIII are associated with the inflammatory response.[23] CKD patients are commonly associated with changes in the levels of various inflammatory cytokines.[24] Proinflammatory substances can activate procoagulant factors and result in elevated levels of particular hemostatic factors.

TEG displays blood clot formation dynamics from initial thrombin generation to fibrinolysis.[25] In the current study, we also performed TEG for dynamic observation of the entire coagulation process in CKD patients. Prior to adjustment for

confounding factors, the TEG data suggested that all aspects of coagulation were increased in the CKD patients, including initial fibrin formation, fibrin-platelet interactions, and qualitative platelet functions. However, after adjustment for relevant influencing factors, we found no significant differences in the TEG parameters (R, K, MA, and angle) between the CKD patients and the healthy controls, which is in contrast to previous TEG studies in hemodialysis patients with ESRD.[26, 27] Hemodialysis patients are influenced by hemodynamic factors and coagulant use and thus present more complicated changes in coagulation functions, which are different from those in non-dialysis CKD patients.[5] Thus, whether TEG can be used to effectively evaluate the integrated coagulation function in non-dialysis CKD patients requires further validation.

In this study, the cardiovascular event occurred in one patient with CKD 5 stage during the one-year of follow-up. This clinical outcome may not be consistent with previous HOPE study which includes 980 subjects and shows 22.2% cumulative incidence of cardiovascular events[28]. However, it should be noted that patients in HOPE study are older (at least 55 years of age) and have higher cardiovascular risk compared with our participants. Besides, the follow-up time (3.5 to 5.5 years) is much longer than that of our current study. The small sample size and short term follow-up in our study might underestimate the risk of thromboembolic events and make it difficult to link the observations with clinical outcomes. Further longer-term study with large samples are still required to more precisely determine the relationship between the elevation of procoagulant factors and clinical outcomes.

The present study has certain limitations. One limitation of this study is the limited number of individuals in the different patient groups and the short term of follow-up. We could not fully evaluate the thromboembolic events. Thus, it limited the applicability of the conclusion of this study. Second, the CKD groups were heterogeneous with a number of factors and complications interfering with the delicate system of hemostasis, even though we had adjusted the related factors and also assessed the hemostatic profiles in small number of participants without the comorbidities.

Conclusions

In conclusion, CKD patients are characterized by endothelial dysfunction and increased coagulation, especially FVIII activity. The abnormal hemostatic profiles may contribute to the elevated risk of thrombotic events and further longer-term study with large samples are still required to more precisely determine the relationship between the elevation of procoagulant factors and clinical outcomes. TEG detected no changes in the coagulation function among the CKD patients. Whether TEG can effectively evaluate the integrated coagulation function in CKD patients needs to be verified using larger samples. Future studies are required to target the role of coagulation management for CKD patients to reduce co-morbidities.

Contributors: H-MJ. W-RB and C-XM created and designed this study. H-MJ. W Y. S-TY. L-QP and Y X collected and analyzed the data. H-MJ. W-RB. D P and L P contributed to the preparation and edition of the manuscript.

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Competing interests: We declare that the authors do not have any potential conflicts of interest.

Data sharing: No additional data

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Figure 1. The flow chart of this study

Figure 2. TEG parameters in healthy controls and chronic kidney disease patients.

(A) R value. (B) K value. (C) MA value. (D) α-angle value.

[#]P-values for the adjusted model. Data are adjusted for age, sex, history of diabetes, history of CHD, smoking status, MAP, BMI, hemoglobin, serum albumin, cholesterol, triglyceride, and UACR.

*p<0.05, vs control group for unadjusted values; ◆p<0.05, vs CKD 5 group for unadjusted values.

Figure 3. Correlation of vWF Ag, vWF:RCo, and FVIII levels with eGFR.

(A) Correlation of vWF Ag with eGFR. (B) Correlation of vWF:RCo with eGFR. (C) Correlation of FVIII with eGFR. Regression lines: (A) vWF Ag=186.3–1.12×eGFR. (B) vWF:RCo=174.2−1.05×eGFR. (C) FVIII=143.2-0.52×eGFR.

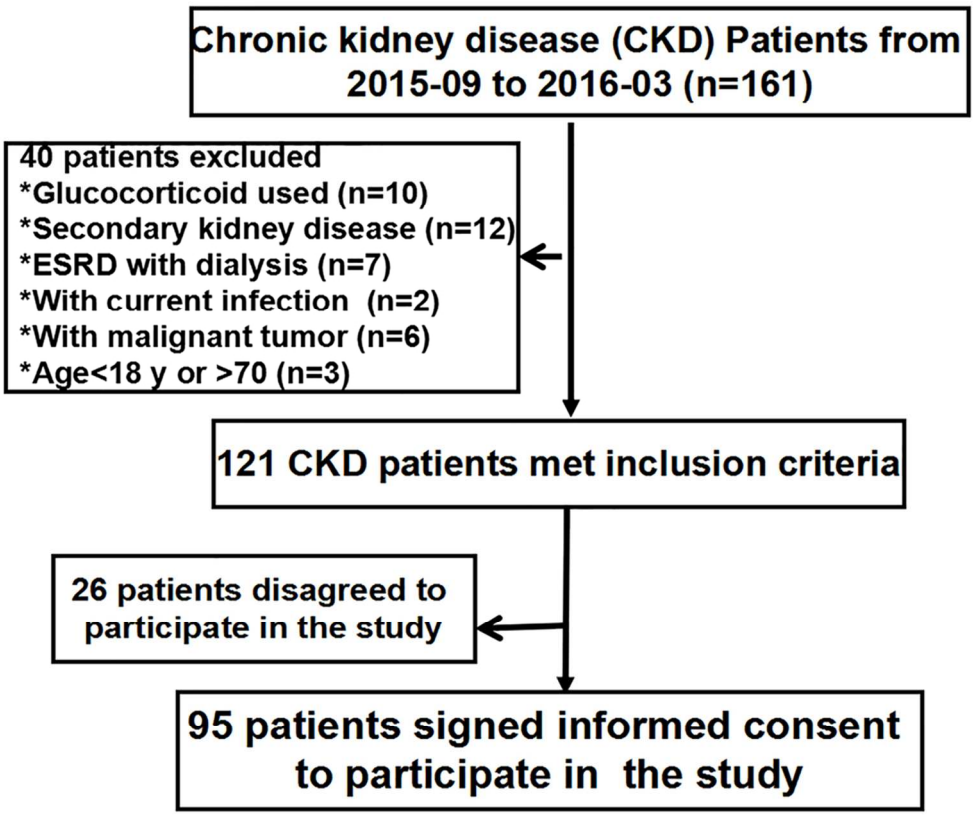

Figure 1. The flow chart of this study

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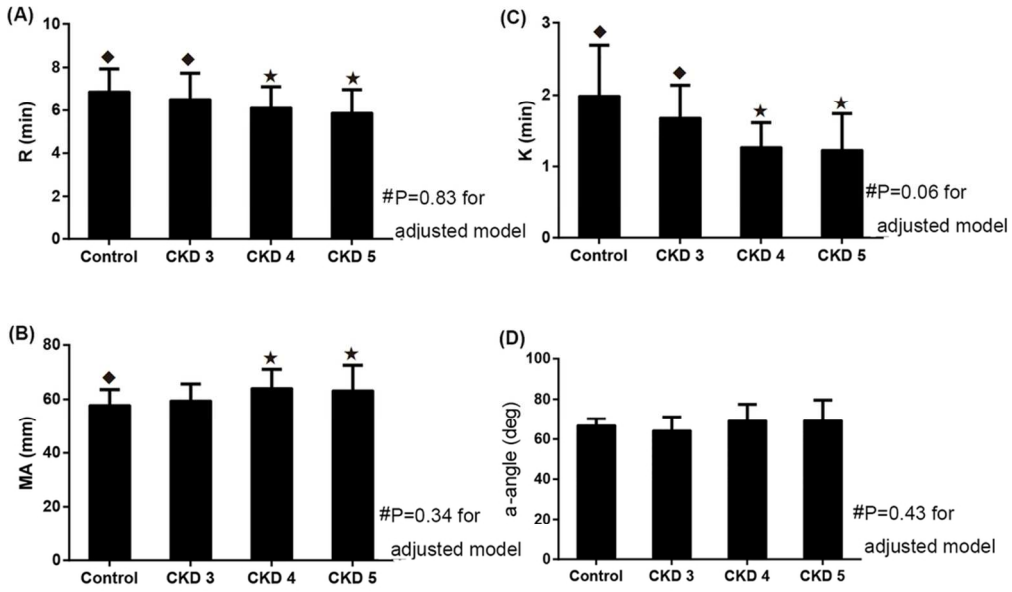

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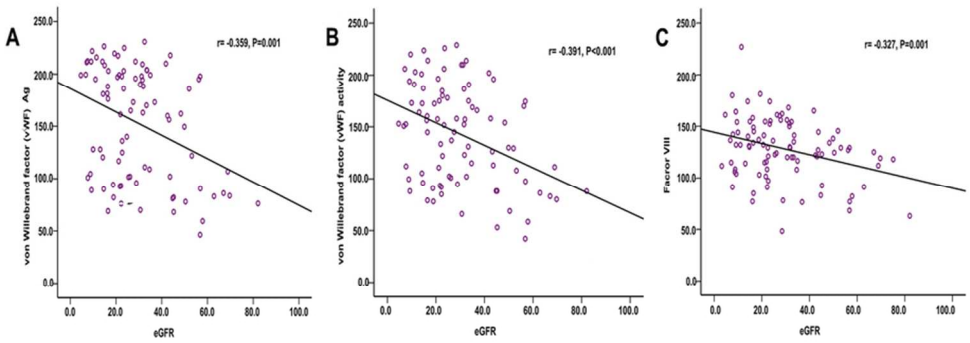

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79x31mm (300 x 300 DPI)

Table S1. Adjusted levels of procoagulant biomarkers by chronic kidney disease status.

Variables	Healthy control	CKD3	CKD4	CKD5
Platelet (10^9/l)	239.3±72.4	218.1±60.5	211.4±57.9	186.3±71.9
ADP $_{LTA}$ (%)	68.2±10.5	68.4±10.1	69.3±2.0	73.6±12.2
Factor V (%)	108.3±38.5	99.7±35.0	108.0±32.6	99.6±40.3
Factor VII(%)	78.8±25.2	99.4±23.4	104.0±20.9	97.7±27.4
Factor VIII(%)	82.3±31.6	118.2±28.9	129.7±27.1	141.1±32.8
VWF:Ag(%)	92.9±51.4	131.0±51.1	155.8±46.2	182.9±56.4
vWF:RCo(%)	86.4±44.1	120.9±44.1	147.9±40.0	170.0±48.8
Fibrinogen(g/l)	3.7±1.0	3.2±0.9	3.7±0.9	4.2±1.0
Protein C(%)	98.2±19.2	97.1±16.6	92.5±15.5	93.3±19.6
Protein S(%)	83.7±29.1	87.1±25.8	93.4±23.5	99.4±30.0
AT III (%)	99.2±12.9	104.7±11.3	104.2±10.4	99.2±12.9
D-dimer,(ng/ml)	362±404	464±367	505±345	780±422
APTT(s)	40.5±4.8	37.8±3.9	37.5±3.6	38.2±4.8
PT(s)	137±0.7	13.5±0.5	13.5±0.6	13.8±0.7

Table S2. Unadjusted and adjusted levels of procoagulant biomarkers in patients without comorbidities

Variables	Unadjusted					Multivariable-adjusted*				
	Healthy control	CKD3 (n=23)	CKD4 (n=29)	CKD5 (n=21)	P	Healthy control	CKD3	CKD4	CKD5	P
Platelet (10^9/l)	231.8±42.7	211.4±65.5	210.6±48.3	195.9±55.4	0.119	231.8±71.8	213.8±58.5	208.6±56.5	196.1±72.9	0.775
ADP $_{LTA}$ (%)	64.3±5.2	64.9±6.4	71.5±8.1	74.8±8.5	0.009	64.4±10.2	65.5±9.1	70.7±8.2	75.6±11.7	0.255
Factor V (%)	108.7±25.4	98.1±35.0	101.6±35.3	99.4±34.7	0.533	101.5±45.6	98.4±41.2	104.1±33.4	98.0±58.1	0.893
Factor VII(%)	77.3±18.9◆	97.9±16.9*◆	103.1±16.6*	108.6±29.1*	0.002	82.7±25.8◆	102.6±22.9*	103.0±21.1*◆	98.3±28.8*	0.139
Factor VIII(%)	88.5±23.8◆	114.7±26.6*◆	126.7±27.4*	136.2±33.5*	<0.001	82.4±33.3◆	117.3±29.5*◆	126.8±27.4*	137.4±34.4*	0.006
VWF:Ag(%)	105.1±45.4◆	124.1±55.8◆	157.3±49.3*	177.0±47.8*	0.002	94.6±54.3◆	127.9±52.7*◆	155.6±47.4*	180.6±58.4*	0.037
VWF:RCo(%)	100.0±32.3◆	114.7±46.4◆	148.1±45.4*	167.5±44.7*	0.001	87.6±47.4◆	118.8±45.8*◆	147.7±41.4*	170.0±51.2*	0.016
Fibrinogen(g/l)	3.0±0.8◆	3.0±0.7◆	3.6±0.6*◆	4.3±1.1*	<0.001	3.5±1.1◆	3.2±0.9*◆	3.5±1.0◆	4.1±0.9*	0.032

Protein C(%)	106.3±17.9◆	98.5±20.3◆	90.4±16.5	86.5±14.9*	0.029	93.4±20.6	96.2±18.3	89.9±16.5	93.7±21.6	0.629
Protein S(%)	76.9±24.8	86.7±25.3	92.8±18.0	97.3±25.4	0.171	81.2±29.3	86.1±25.0	91.2±23.5	98.6±30.0	0.651
AT III (%)	100.9±8.9	103.6±10.9	106.1±10.4	103.7±12.2	0.539	97.8±12.0	104.2±10.0	105.9±9.6	102.2±12.5	0.178
D-dimer,(ng/ml)	268±124◆	419±297◆	420±186*◆	865±531*	<0.001	373±300◆	464±335	419±323	799±40*	0.008
APTT(s)	38.9±4.8	37.9±3.4	37.9±3.9	39.4±3.3	0.506	41.2±4.7	38.2±3.8	37.6±3.8	38.1±4.5	0.138
PT(s)	13.5±0.6	13.6±0.6	13.5±0.6	13.7±0.5	0.655	13.6±0.7	13.5±0.6	13.5±0.6	13.6±0.7	0.920

Data are adjusted for age, sex, MAP, BMI, hemoglobin, serum albumin, cholesterol, triglyceride, and UACR.

AT III=antithrombin III; APTT=activated partial thromboplastin time; PT=prothrombin time; LTA=Light transmittance aggregometry.

*p<0.05, vs control group; ◆p<0.05, vs CKD 5 group.

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 4
Objectives	3	State specific objectives, including any pre-specified hypotheses	Page 4
Methods			
Study design	4	Present key elements of study design early in the paper	Page 5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 5-7
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Page 5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 5-7
Bias	9	Describe any efforts to address potential sources of bias	Page 8
Study size	10	Explain how the study size was arrived at	Page 5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 6,7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 8
		(*b*) Describe any methods used to examine subgroups and interactions	Page 8
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	Page 8

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Page 8,9
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	Page 6
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 8,9
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	Page 14
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 11-15
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 16
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 16-18
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 19

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.