PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Blood coagulation system in patients with chronic kidney disease: a prospective observational study
AUTHORS	Huang, Meng-Jie; Wei, Ri-bao; Wang, Yang; Su, Ting-yu; Di, Ping; Li, Qing-ping; Yang, Xi; Li, Ping; Chen, Xiang-mei

VERSION 1 - REVIEW

REVIEWER	ND Vaziri MD, MACP UC Irvine California, USA
REVIEW RETURNED	30-Sep-2016

GENERAL COMMENTS	In this study the authors have measured several important coagulation factors in subgroups of patients with different stages of CKD. They found significant elevations of the vWF:Ag, vWF:RCo, fibrinogen, FVII, FVIII, and D-dimer levels in the CKD patients compared with those found in the healthy controls. The magnitude of elevation of the given parameters was proportional to severity of CKD. Based on these observations they conclude that CKD patients exhibit endothelial dysfunction and enhanced coagulation, and that the abnormal hemostatic profiles may contribute to the elevated risk of thrombotic events in CKD population. The study is well designed and executed and the results are of interest. I can therefore recommend acceptance of their paper for publication in BMJ.

REVIEWER	Prof. Dr. Jens Lutz Universitätsmedizin Mainz Germany
REVIEW RETURNED	22-Dec-2016

GENERAL COMMENTS	The paper from Huang et al. analyses the coagulation system in patients with chronic kidney diseases which is an important clinical Problem in These patients. However, the results are not entirely new and have been reported in earlier publications (i.e. Remuzzi G. et al.). In particular it is important to exclude smokers as well as patients with diabetes mellitus and arterial hypertension that is not well controlled as all These factors influencce the coagulation system. Furthermore, the authors should try to link their observations with clinical outcomes such as thrombotic events as this would help to understand why some patients experiience such events to a greater extent than others. Furthermore, direct analysis of platelets should be included to give a clear Picture on the coagulation system in patients with chronic kidney diseases.

REVIEWER	Maria Fusaro CNR-IFC and University of Padua, Italy
REVIEW RETURNED	29-Jan-2017

GENERAL COMMENTS	The Manuscript Blood coagulation system in patients with chronic Kidney disease: a prospective observational study is suitable for publication. However Authors are strongly advised to improve Table 2.

REVIEWER	Jonas Spaak Department of Cardiology and Clinical Sicences, Danderyd University Hospital, Karolinska Institutet. Sweden.
REVIEW RETURNED	03-Feb-2017

GENERAL COMMENTS	This is a prospective cohort study of haemostatic markers in relation to renal function. The study is only descriptive but fills a knowledge gap, since this kind of data has not been reported previously. The methodology is sound and the presentation straightforward. The readability could be improved by omitting the adjusted values in table 3 and fig 3, and potentially moved to an appendix. Minor comments: Primary subject heading: urology – probably better with nephrology or hemostasis. Abstract first section ”underlying thrombophilia”, a term usually used in coagulation defects, please change to ”of their high throboembolic risk” or ” prothrombotic state” throughout the text. Abstract conclusion, please change ”enhanced coagulation” to ”increased coagulation”, as the word enhanced usually implies something positive. Same for the conclusion at the end of the discussion. Table 2 – multivariable adjusted is useful for the statistical analysis, but not necessary to provode individual values, ie keep unadjusted values and add p-values for the adjusted model but skip the actual values. The same applies for the thromboelastography data, that would be better presented as the unadjusted values and just add the statistics (p-values) for the adjusted model, ie fig Table 3 could be omitted and that info added to results and to the legend of fig 3.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 2
(A) However, the results are not entirely new and have been reported in earlier publications (i.e. Remuzzi G. et al.)

Response: We are very sorry for the insufficient innovation. We found that previous studies on the role of hemostatic factors in CKD are mostly limited to people with mild renal dysfunction (eGFR≥90; 90>eGFR≥60; 60>eGFR ≥30 ml/min per 1.73 m2) or end-stage renal disease requiring dialysis. But no similar reports are available involving people with moderate-to-severe renal insufficiency. In our

study, we defined the CKD as an estimated GFR decreasing to less than 60 mL/min/1.73 m2, representing stages 3 to 5 of CKD and excluded dialysis patients.
Besides, based on the analysis of platelets, vascular endothelium, coagulation system, anticoagulant system, standard coagulation tests and thromboelastography, we hope to give a clear picture on the coagulation system in patients with chronic kidney diseases.

(B) In particular it is important to exclude smokers as well as patients with diabetes mellitus and arterial hypertension that is not well controlled as all. These factors influence the coagulation system.

Response: We acknowledge your comment and suggestion very much, which is valuable in improving the preciseness of our manuscript. Following your advice, we excluded smokers as well as patients with diabetes mellitus and arterial hypertension that were not well controlled and then compared the procoagulant markers among these patients with different stages of CKD. The results showed that the positive associations of renal insufficiency with these hemostatic profiles were similar in participants with or without the above-mentioned comorbidities.
As this prospective study was conducted from September 2015 to March 2016, we were unable to include new participants at this time. Thus when we excluded smokers as well as patients with diabetes mellitus and uncontrolled hypertension, only a few participants were left. Due to the limited sample size, low proportion of diabetes, smoking and uncontrolled hypertension, as well as the similar hemostatic results in patients with and without comorbidities, our study contained both of these results (patients with and without comorbidities) and we presented the detailed hemostatic information of patients without comorbidities on page 11, paragraph 2 and on Supplementary material Table S2.

(C) Furthermore, the authors should try to link their observations with clinical outcomes such as thrombotic events as this would help to understand why some patients experience such events to a greater extent than others.

Response: We appreciate and agree with this important suggestion. Based on your suggestion, we recorded the incidence of thromboembolic events in the CKD patients and healthy controls during the one-year of follow-up (since all the patients were included between September 2015 and March 2016). 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.
The follow-up records showed that 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. However, the small sample size and short term follow-up might underestimate the risk of thromboembolic events.
The detailed information was presented on page 8, paragraph 2; page 14, paragraph 1, and page 16, paragraph 2.

(D) Furthermore, direct analysis of platelets should be included to give a clear picture on the coagulation system in patients with chronic kidney diseases.

Response: We are very sorry for our negligence of the platelet function analysis. As our center started to carry out the assessment of platelet aggregation on January 2016, just part of the included patients underwent this test. In fact, 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.
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 250×109/L. Platelet aggregability was assessed at 37°C 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.
The analysis showed that platelet aggregability was significantly higher in the CKD 5 patients than in the controls (Control vs CKD3 vs CKD4 vs CKD5: 64.6±4.8 vs 67.3±8.6 vs 70.1±8.6 vs 74.7±8.2; P=0.041); however, after adjustment, no significant difference was found in this parameter between groups (P=0.738).
The detailed information was added on page 7, paragraph 1 and on Table 2.

Reviewer: 3
The Manuscript Blood coagulation system in patients with chronic Kidney disease: a prospective observational study is suitable for publication.
However Authors are strongly advised to improve Table 2.

Response: We followed this suggestion and rearranged Table 2. In order to make the Table 2 simple and clear, we kept unadjusted values, add p-values for the adjusted model but moved the adjusted levels of procoagulant biomarkers to Supplementary Table S1.
Table 2. Procoagulant biomarkers by chronic kidney disease status.

Variables	Healthy control	CKD3	CKD4	CKD5	P	#P
Platelet (109/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

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

Variables	Healthy control	CKD3	CKD4	CKD5
Platelet (109/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

Reviewer: 4
The readability could be improved by omitting the adjusted values in table 3 and fig 3, and potentially moved to an appendix.

Response: We appreciate and agree with this important suggestion. But we were a little confused that whether you were referring to table 2 and figure 2. As table 3 was the multivariable-adjusted regression coefficients of hemostatic biomarkers with eGFR and figure 3 was the correlation of vWF Ag, vWF:RCo, and FVIII levels with eGFR, there were no adjusted values in table 3 and fig 3. Thus we rearranged Table 2 and Figure 2 by omitting the adjusted values and moved this data to an appendix.

Minor comments:
Primary subject heading: urology-probably better with nephrology or hemostasis.
Abstract first section “underlying thrombophilia”, a term usually used in coagulation defects, please change to “of their high throboembolic risk” or “prothrombotic state” throughout the text.

Response: We corrected and changed these terms to "of their high throboembolic risk" throughout the manuscript. Thank you for your effort.

Abstract conclusion, please change “enhanced coagulation” to “increased coagulation”, as the word enhanced usually implies something positive. Same for the conclusion at the end of the discussion.

Response: We are very sorry for our inappropriate statement of the “enhanced coagulation”. Following your advice, we have modified the term into “increased coagulation” in the abstract and discussion section.

Table 2-multivariable adjusted is useful for the statistical analysis, but not necessary to provide individual values, ie keep unadjusted values and add p-values for the adjusted model but skip the actual values.

Response: We followed your suggestion and rearranged Table 2 by deleting the adjusted values and keeping p-values for the adjusted model. Besides, we presented the adjusted levels of procoagulant biomarkers on Supplementary Table S1.

Table 2. Procoagulant biomarkers by chronic kidney disease status.
Variables Healthy control CKD3 CKD4 CKD5 P #P
Platelet (109/l) 237.2±47.4 214.8±65.0 214.0±52.8 195.8±58.6 0.156 0.284
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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.
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PT(s) 137±0.7 13.5±0.5 13.5±0.6 13.8±0.7

The same applies for the thromboelastography data, that would be better presented as the unadjusted values and just add the statistics (p-values) for the adjusted model, ie fig

Response: Following your advice, we deleted the adjusted values of thromboelastography and just add their statistics (p-values) in Figure 2. Thank you for your effort.

Table 3 could be omitted and that info added to results and to the legend of fig 3.

Response: We have deleted the Table 3 and added the multivariable linear regression information “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, 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].” to the results. The detailed information was presented on page 13, paragraph 2
Besides, we also added the 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.” to the legend of Figure 3.

VERSION 2 – REVIEW

REVIEWER	Jonas Spaak Department of clinical sciences, Danderyd University Hospital, Karolinska Instutet, Sweden
REVIEW RETURNED	11-Mar-2017

GENERAL COMMENTS	In my opinion ready for publication. Best/Jonas