Role of Hemostatic Factors on the Risk of Venous Thrombosis in Persons with Impaired Kidney Function

Running title: Ocak et al.; Kidney function and venous thrombosis

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Journal Subject Codes: Thrombosis:[173] Deep vein thrombosis, Thrombosis:[174] Coagulation
Abstract

Background—Factors explaining the association between impaired kidney function and venous thrombosis have not been identified so far. The aim of our study was to determine whether the association between impaired kidney function and venous thrombosis can be explained by the concurrent presence of genetic or acquired venous thrombosis risk factors.

Methods and Results—The glomerular filtration rate was estimated (eGFR) in 2473 venous thrombosis patients and 2936 controls from a population-based case-control study. Kidney function was grouped into 6 categories based on percentiles of the eGFR in the controls (>50th percentile [reference], 10th-50th percentile, 5th-10th percentile, 2.5th-5th percentile, 1st-2.5th percentile, and <1st percentile). Several hemostatic factors showed a procoagulant shift with decreasing kidney function in controls, most notably factor VIII and von Willebrand factor (VWF). As compared with eGFR > 50th percentile, factor VIII levels (adjusted mean difference of 60 IU/dl for the <1st eGFR percentile category) and VWF levels (adjusted mean difference of 60 IU/dl for the <1st eGFR percentile category) increased with each percentile category. The ORs for venous thrombosis similarly increased across the categories from 1.1 (95%CI, 0.9-1.3) for the 10th-50th percentile to 3.7 (95%CI, 2.4-5.7) for the <1st percentile category. Adjustment for factor VIII or von Willebrand factor attenuated these ORs indicating an effect of eGFR on thrombosis through these factors. Adjustments for other risk factors for venous thrombosis did not affect the ORs.

Conclusions—Impaired kidney function affects venous thrombosis risk via concurrently raised factor VIII and von Willebrand factor levels.

Key words: chronic kidney disease, venous thromboembolism
Introduction

The overall incidence of venous thrombosis is 1-2 per 1000 persons each year which rises exponentially with age, from 0.005% in children to 1% per year in the elderly.\(^1\) The prevalence of kidney disease is increasing due to ageing and a concurrent rise in prevalence of diabetes,\(^2\) which explains the growing interest in the role of kidney disease as a risk factor for venous thrombosis.\(^3\) Several population-based studies have shown that chronic kidney disease increases the risk of venous thrombosis.\(^4,5\)

Unfortunately, studies that described this association were limited in providing information on explanatory factors. Knowledge of these mechanisms is important, both from a clinical as from a scientific viewpoint. The association between chronic kidney disease and venous thrombosis might be explained by the presence of common risk factors (confounders) that are associated with both venous thrombosis and chronic kidney disease, such as an increased body mass index,\(^6,7\) factor V Leiden,\(^8,9\) prothrombin G20210A,\(^8,9\) diabetes mellitus,\(^6,10\) malignancy,\(^9,11\) and arterial thrombosis.\(^12,13\) The association might also be explained by factors that are a consequence of chronic kidney disease (mediators), which in their turn increase the risk of venous thrombosis such as immobilization,\(^9\) surgery,\(^9\) corticosteroid use,\(^14\) or changes in hemostatic factors.\(^9\)

The aim of our study was therefore to investigate whether the association between impaired kidney function and venous thrombosis can be explained by potential confounders and mediators. To this aim, we measured the estimated glomerular filtration rates (eGFRs) in 2473 patients with a recent venous thrombosis and 2936 matched control subjects participating in a large population-based case-control study (MEGA study).
Methods

Study design

The MEGA study (Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis study) is a large, population-based case-control study on risk factors for venous thrombosis. Between March 1999 and September 2004, consecutive patients aged 18 to 70 years with a first objectively confirmed episode of deep venous thrombosis or pulmonary embolism were included from six participating anticoagulation clinics in the Netherlands. Information on the diagnostic procedure was obtained from hospital records and general practitioners. This study was approved by the Ethics Committee of the Leiden University Medical Center and written informed consent was obtained from all the participants. The investigation has been conducted according to the principles expressed in the Declaration of Helsinki.

Study subjects

Only patients with a diagnosis of venous thrombosis that was confirmed with objective techniques were included in the analyses, as previously described. Exclusion criteria were severe psychiatric problems and inability to speak Dutch. Of the 6567 eligible thrombosis patients, 5183 participated (79%). For logistic reasons, blood sampling was performed for participants included up to June 2002 (n=2473) (Figure 1). Two sets of controls were gathered; partners of patients and subjects from the general population reached by random digit dialing (RDD). Of the 3735 partner controls (age <70 years without venous thrombosis), 3297 participated and 1480 provided blood samples (Figure 1). Of 5183 RDD controls (frequency matched to patients on age and sex) without venous thrombosis who were approached via a random-digit-dialing (RDD) method (recruited from the same geographical area as the patients), 4350 were eligible, 3000 participated and 1456 provided blood (Figure 1). Of the 1480 partner
controls, 1316 partner controls could be matched with a thrombosis patient, i.e. of 164 partners the corresponding patient originally participated, but was later found not to be eligible (aged over 70 years, not objectivated thrombosis, or not a first thrombotic event). These control subjects were included in the overall analyses but not in the matched patient-partner analysis.

**Data collection**

All persons were asked to complete an extensive questionnaire on many potential risk factors for venous thrombosis. Of interest for the current analysis are the items on general health characteristics, immobilization, surgery, a history of arterial thrombosis (angina, myocardial infarction, ischemic stroke, peripheral vascular disease, or transient ischemic attack), malignancy, diabetes mellitus, and corticosteroid use. The index date was the date of the thrombotic event for patients and their partners, and the date of filling in the questionnaire for the random controls.

**Laboratory assays**

Approximately 3 months after discontinuation of oral anticoagulant therapy, thrombosis patients and their partners were invited for collection of a blood sample. In patients who were still on anticoagulant therapy 1 year after their event, blood was drawn during anticoagulant therapy. Serum creatinine was measured enzymatically (Roche Diagnostics, Mannheim, Germany). Glomerular filtration rate was estimated by the Modification of Diet in Renal Disease (MDRD) study equation. The common genetic risk factors factor V Leiden and prothrombin G20210A were determined using the TaqMan assay. Levels of natural anticoagulants (antithrombin, protein S and protein C levels) and procoagulant factors (fibrinogen, factor II, factor VII, factor VIII, von Willebrand factor, factor IX, factor X, and factor XI) were also assessed. All assays were performed in automated machines by laboratory technicians who were unaware of the case-
control status of the samples. A detailed description of how these laboratory markers were analyzed has been published previously.17-20

Statistical analysis

Hemostatic factor levels in controls in relation to kidney function

We investigated whether impaired kidney function was associated with changes in hemostatic factors in controls. Kidney function was grouped into 6 categories based on percentiles of the eGFR of the controls (>50th percentile (reference), 10th -50th percentile, 5th -10th percentile, 2.5th – 5th percentile, 1st – 2.5th percentile, and <1st percentile). The reason to use these 6 percentile groups was to investigate a wide range of eGFR regions, particularly for the abnormal levels. We calculated age and sex adjusted mean differences with 95% confidence intervals (95% CIs) in levels of hemostatic factors for 10th -50th percentile, 5th -10th percentile, 2.5th – 5th percentile, 1st – 2.5th percentile, and <1st percentile of the kidney function in control persons as compared with the >50th percentile using linear regression. Furthermore, we used linear regression to calculate the decrease or increase in levels of hemostatic factors in control persons for every increase of 10 ml/min in eGFR after adjustment for age and sex.

Case-control comparisons: risk of venous thrombosis and eGFR

To determine whether an impaired kidney function was associated with an increased risk for venous thrombosis, age and sex adjusted odds ratios with 95% CIs were calculated as estimates of the relative risk for the different levels of eGFR. In addition, we adjusted for potential confounding and mediating factors to explore whether an increased risk was explained by these factors. The following potential confounders were included in the model: body mass index, factor V Leiden, prothrombin G20210A, diabetes mellitus, malignancy, and arterial thrombosis including angina, myocardial infarction, ischemic stroke, transient ischemic attack, and
peripheral vascular disease. Subsequently we included factors that might mediate the increased risk of VT associated with chronic kidney disease, i.e., immobilization, surgery, and corticosteroid use. Finally, we adjusted for the hemostatic factors (as continuous variables) (Figure 2). Lastly, we re-analyzed the data using clinical cut-off points instead of percentiles for kidney function (normal kidney function (eGFR>90 ml/min), mildly decreased kidney function (eGFR 60-90 ml/min), and moderately to severely decreased kidney function (eGFR <60 ml/min). As a sensitivity analyses, we applied the CKD-EPI instead of the MDRD equation. We ran three parallel analyses to determine the direction of the precision of the association, 1) patients compared to the pooled control groups, 2) patients compared to partner controls (conditional logistic regression), and 3) patients compared to RDD controls (unconditional logistic regression). The first analysis used non-conservative estimates of the standard errors, whereas the second and third analyses provide overly conservative estimates when applied to the pooled analysis.

Case-case comparisons: effect of time between venous thrombosis and blood sampling on eGFR mean levels

In addition, we compared mean eGFRs of patients who were tested within 3-6 months, 6-12 months, or > 12 months after their first venous thrombosis (ANOVA test). Statistical analyses were performed with statistical package SPSS Windows version 17.0 (SPSS, Chicago, IL, USA).

Results

Table 1 shows the baseline characteristics of the study population. In total, 5183 patients and 6297 control persons (3297 partner controls and 3000 RDD controls) participated in the MEGA study. Of the patients, 2473 provided blood samples. Of the control persons, 2936 provided
blood samples. There were no substantial differences in the baseline characteristics in all participants as compared with participants that provided blood samples (Table 1). Of the 2473 thrombosis patients, 1473 (59.6%) had a deep vein thrombosis only and 1000 (40.4%) had a pulmonary embolism with or without deep vein thrombosis. There were negligible age or sex differences between patients and controls. Body mass index was higher in thrombosis patients than in controls. Furthermore, compared with controls, venous thrombosis patients more often used corticosteroids, more often were carriers of factor V Leiden or prothrombin G20210A and more often had a history of arterial thrombosis, malignancy, diabetes mellitus, immobilization or surgery. There were no substantial differences between the partner and RDD controls in the baseline characteristics (supplementary Table 1).

Hemostatic factor levels in controls in relation to kidney function

In control participants, several hemostatic factors showed a shift towards a procoagulant state with decreasing kidney function (Table 2). Compared to subjects with an eGFR >50th percentile, the adjusted mean factor levels were significantly different from subjects with an eGFR <1st percentile for fibrinogen (adjusted mean difference 0.7 g/L; 95% CI 0.5-0.9), factor VII (31 IU/dL; 95% CI 22-40), factor IX (12 IU/dL; 95% CI 5-19), and factor XI (10 IU/dL; 95% CI 3-17), with a most pronounced increase for factor VIII (60 IU/dL; 95%CI 44 to 76) and von Willebrand factor (60 IU/dL; 95%C 43 to 77). A decrease of 10 ml/min in eGFR was associated with a an increase of 3 IU/dL (95% CI 2 to 4) in factor VIII levels and an increase of 2 IU/dL (95% CI 1 to 3) in von Willebrand factor levels. The results were the same when clinical cut-off points were used to categorize kidney function instead of percentiles. Persons with moderately to severely decreased kidney function (eGFR <60 ml/min) had procoagulant changes as compared with persons with normal kidney function (eGFR >90 ml/min) (Table 3), most
notably in levels of factor VIII (41 IU/dL; 95% CI 31-51) and von Willebrand factor (32 IU/dL; 95% CI 21-43). These results were in the same range for partner controls and for RDD controls.

**Case-control comparisons: risk of venous thrombosis and eGFR**

**Table 4** shows the risk of venous thrombosis for categories of eGFR. As compared with subjects in the >50th percentile, decreasing eGFR was associated with a steadily increasing risk, i.e. from a 1.1-fold (95% CI 0.9-1.2) increased risk for subjects in the 10th – 50th percentile to a 3.7-fold (95% CI 2.4-5.7) increased risk in subjects with an eGFR <1st percentile. Adjustment for potential confounders (body mass index, diabetes mellitus, arterial thrombosis, malignancy, prothrombin G20210A, and factor V Leiden) slightly attenuated these risk estimates. Additional adjustment for potential mediators between impaired kidney function and venous thrombosis (immobilization, surgery, and corticosteroid use) further decreased this risk slightly. After additional adjustment for factor VIII and von Willebrand factor levels, i.e. the two hemostatic factors that showed the strongest relation with impaired kidney function, the odds ratios attenuated to almost unity in all percentiles. Additional adjustment for other hemostatic factors did not further alter the odds ratios. **Figure 3** shows the risk of venous thrombosis for different percentiles of kidney function after adjustment for factor VIII and von Willebrand factor levels only (without adjustment for the other possible mediators and confounders). For factor VIII levels, participants with levels >150 IU/dL had an 8.0-fold (95% CI 6.7-9.5) increased risk of venous thrombosis as compared with participants with levels <100 IU/dL. Results were in the same direction and risks similarly attenuated after adjustment for the coagulation factors when both control groups were analyzed separately (**supplementary Table 3**).

The results were the same when clinical cut-off points were used to categorize kidney function instead of percentiles. Moderately to severely decreased kidney function was associated
with a 2.6-fold (95% CI 2.0-3.5) increased risk of venous thrombosis as compared with normal kidney function in the pooled results, with a 3.8-fold (95% CI 2.4-6.0) increased risk versus the partner controls and with a 2.2-fold (95% CI 1.6-3.2) increased risk versus the RDD controls after adjustment for age and sex. Odds ratios for moderately to severely decreased kidney function were again attenuated to the null after adjustment for von Willebrand factor and factor VIII levels (odds ratio 1.2, 95% CI 0.8-1.7 for the combination of both coagulation proteins, odds ratio of 1.4 (95% CI 0.9-2.0) for von Willebrand factor only and odds ratio of 1.2 (95% CI 0.8-1.7) for factor VIII only). Both in the partner controls and RDD controls, odds ratios attenuated for moderately to severely decreased kidney function and venous thrombosis after adjustment for von Willebrand factor and factor VIII levels (Table 5).

Results were in the same direction and risks attenuated after adjustment for the coagulation factors when both control groups were analyzed separately (Table 5). Furthermore, as the MDRD equation may underestimate glomerular filtration rates at borderline abnormal levels (i.e., 60mL/minute), while most of the participants with reduced kidney function were close to this level, it is possible that reclassification of kidney function by the CKD-EPI equation gives more valid results. However, as Table 6 shows, both equations led to similar results.

**Case-case comparisons: effect of the time between venous thrombosis and blood sampling on eGFR mean levels**

No major differences in mean eGFRs were observed when patients were tested within 3-6 months (mean 87 ml/min), 6-12 months (mean 86 ml/min), or > 12 months (mean 86 ml/min) after their first venous thrombosis.

**Discussion**

In this large population-based case-control study, an association was found between impaired
kidney function and levels of fibrinogen, factor VII, factor IX, factor XI, and of factor VIII and von Willebrand factor levels. Furthermore, the increased risk of venous thrombosis with decreasing kidney function seemed fully explained by concurrently raised levels of factor VIII or von Willebrand factor.

Both in the LITE\textsuperscript{4} and PREVEND\textsuperscript{5} study chronic kidney disease was associated with an increased risk of venous thrombosis. However, in neither study, factors that might explain the association between chronic kidney disease and venous thrombosis were identified. Our analyses showed that presence of common risk factors for chronic kidney disease and venous thrombosis, such as body mass index\textsuperscript{6,7} factor V Leiden\textsuperscript{8,9} prothrombin G20210A,\textsuperscript{8,9} diabetes mellitus\textsuperscript{6,10} malignancy,\textsuperscript{9,11} and arterial thrombosis\textsuperscript{12,13} could not explain the association. In our attempt to explain the increased risk of venous thrombosis in chronic kidney disease, we also adjusted for risk factors that are a consequence of chronic kidney disease and increase in their turn the risk of venous thrombosis (mediators), such as immobilization,\textsuperscript{9} surgery,\textsuperscript{9} corticosteroid use,\textsuperscript{14} and changes in hemostatic factors.\textsuperscript{9} Immobilization, surgery, and corticosteroid use only slightly changed the odds ratio. However, factor VIII and von Willebrand factor could fully explain the increased risk of venous thrombosis associated with impaired kidney function.

In previous studies, patients with end-stage renal disease and nephrotic syndrome (defined as proteinuria of more than 3 grams per 24 hours) were shown to have elevated levels of fibrinogen, factor VIII and von Willebrand factor.\textsuperscript{21-23} In addition, patients with nephrotic syndrome have decreased antithrombin levels due to urinary loss of antithrombin.\textsuperscript{24} Increased levels of fibrinogen,\textsuperscript{25,26} factor VIII,\textsuperscript{27} factor IX,\textsuperscript{9} factor XI,\textsuperscript{9} and von Willebrand factor\textsuperscript{28} have been associated with an increased risk of venous thrombosis in the general population, whereas factor VII was not associated with venous thrombosis in previous studies.\textsuperscript{27,29} In our study, we
observed a procoagulant shift in subjects with an impaired kidney function <1st percentile corresponding with an eGFR of <53 ml/min: levels of fibrinogen, factor VII, factor IX and factor XI, and especially levels of factor VIII and von Willebrand factor were increased. We did not find an association between antithrombin levels and impaired kidney function.

We showed that impaired kidney function, either estimated with the MDRD equation or with the CKD-EPI equation, affects venous thrombosis risk via concurrently raised factor VIII and von Willebrand factor levels. However, the exact mechanism through which chronic kidney disease leads to venous thrombosis via procoagulant changes (especially increases in factor VIII and von Willebrand factor levels) cannot be determined from these data with certainty. As von Willebrand factor and factor VIII are markers of endothelial damage, it might be that endothelial damage, which is associated with chronic kidney disease, leads to increased factor VIII and von Willebrand factor levels and eventually to venous thrombosis. According to this view, chronic kidney disease would be an epiphenomenon to the risk of venous thrombosis, and the endothelial damage that leads to a procoagulant shift would be the underlying cause. Alternatively, the endothelial damage could be caused by the chronic kidney disease, which leads to a procoagulant state and finally to venous thrombosis.

The strengths of this study include the large patient sample, the detailed information about genetic and acquired risk factors for venous thrombosis, medication use, and comorbidities in both patients and controls in combination with hemostatic factor level information. In our study, blood was collected after the thrombotic events as a consequence of our study design (case-control study) minimizing the time frame between event and measurements (eGFR and hemostatic factors). Cohort studies have the drawback that they usually assess indicators at baseline, long before the occurrence of the disease, resulting in a possible dilution of the effect,
especially when we take into account that kidney function and hemostatic factors levels could change in the years before the disease. As there is a time lag in cohort studies between the event and assessments (kidney function), case–control studies might be better for showing the association between kidney function and the risk of venous thrombosis. Furthermore, it is unlikely that differences in creatinine levels between cases and control persons were the result of the thrombotic event itself. No major differences in mean eGFRs were observed when patients were tested within 3-6 months, 6-12 months, or > 12 months after their first venous thrombosis suggesting that these levels were not influenced by a temporarily raised effect. In addition, it is not likely that our results are explained by acute phase reactions from the thrombotic event itself, as the clear dose-response relationship between decreased kidney function and increased factor VIII and von Willebrand factor was observed in subjects without venous thrombosis. Furthermore, it is not likely that an acute phase reaction results in higher levels of factor VIII and von Willebrand factor in venous thrombosis patients with chronic kidney disease than in subjects with venous thrombosis and a normal kidney function. Moreover, factor VIII and von Willebrand factor were measured at least 3 months after the venous thrombotic event occurred in patients, thereby minimizing any acute phase reactions.\(^1\) Another potential limitation in our study was that blood was provided in a subset of the participating patients and controls in the MEGA study. However, since we stopped taking blood after June 2002 for logistic reasons only and since baseline characteristics were similar, it is unlikely that this has introduced bias. Lastly, we had no information about proteinuria. It would be useful to explore whether proteinuria is associated with an increased risk of venous thrombosis and whether such an association can be explained by changes in hemostatic factors. Proteinuria, especially in the nephrotic range (defined as proteinuria of more than 3 grams per 24 hours), has been associated with venous
It has been suggested that nephrotic syndrome leads to venous thrombosis through loss of antithrombin in the urine. This, however, was beyond the scope of our study where we aimed to relate eGFR levels to venous thrombosis risk, taking potential confounding and mediation into account. Furthermore, we did not find an association between decreased kidney function and low levels of antithrombin. Another limitation of our study was that we cannot provide risk estimates by the primary kidney disease. This is because most of the subjects with impaired kidney function in our study had no symptoms and were never, or not yet diagnosed with impaired kidney function. It would certainly be useful to study the risks of thrombosis for the various types of primary kidney disease, for which rather than comparing patients with thrombosis to controls, patients with specific kidney disorders should be followed for the development of thrombosis, since these various diseases are each too rare to differentiate in a thrombosis case-control study. A final aspect of our study was that we had two separate control groups. The analysis that pooled controls could have understated the standard errors. A more elaborate analysis that treated the control groups as a random effect would have produced more conservative standard errors. The pooled estimate was a weighted mixture of the separate estimates for each of the two control groups, so, by design, between them. Also, the pooled analysis does not easily generalize to a known population. Nevertheless, results pointed in the same direction and were roughly similar when both control groups were analyzed separately. Therefore, our results were not affected by the use of two different control groups.

In summary, we have reported a detailed epidemiological analysis into the risk of first venous thrombosis in individuals with reduced kidney function. We showed that the increased risk of venous thrombosis can be explained by concurrently raised factor VIII and von Willebrand factor levels.
**Acknowledgments:** The authors thank the directors of the Anticoagulation Clinics of Amersfoort (M. H. H. Kramer), Amsterdam (M. Remkes), Leiden (F. J. M. van der Meer), The Hague (E. van Meegen), Rotterdam (A. A. H. Kasbergen), and Utrecht (J. de Vries-Goldschmeding) who made the recruitment of patients possible. The interviewers (J. C. M. van den Berg, B. Berbee, S. van der Leden, M. Roosen, and E. C. Willems of Brilman) performed the blood draws. The authors also thank I. de Jonge, R. Roelofsen, M. Streevelaar, L. M. J. Timmers and J. J. Schreijer for their secretarial and administrative support and data management. The fellows I. D. Bezemer, J. W. Blom, A. van Hylckama Vlieg, E. R. Pomp and L. W. Tick took part in every step of the data collection. C.M. Cobbaert, C. J. M. van Dijk, R. van Eck, J. van der Meijden, P. J. Noordijk and T. Visser performed the laboratory measurements. We express our gratitude to all individuals who participated in the MEGA study. Dr Lijfering is a Postdoc of the Netherlands Heart Foundation (2011T012). This research was supported by the Netherlands Heart Foundation (NHS 98.113), the Dutch Cancer Foundation (RUL 99/1992) and the Netherlands Organization for Scientific Research (912-03-033 2003).

**Conflict of Interest Disclosures:** The authors declare that they have no conflict of interest.

**References:**


2010;149:118-123.


Table 1. Baseline characteristics

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<th>Patients in MEGA</th>
<th>Patients tested</th>
<th>Controls in MEGA</th>
<th>Controls tested</th>
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<td>N=5183</td>
<td>N=2473</td>
<td>N=6297</td>
<td>N=2936</td>
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<tr>
<td>Median age, years (5-95th %)</td>
<td>49.1 (25.9-67.5)</td>
<td>49.6 (25.5-67.8)</td>
<td>47.7 (25.4-66.6)</td>
<td>49.8 (27.1-67.0)</td>
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<td>Women, n (%)</td>
<td>1346 (54.4%)</td>
<td>2801 (54.0%)</td>
<td>3383 (53.7%)</td>
<td>1543 (52.6%)</td>
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<tr>
<td>BMI, kg/m² (5-95th %)</td>
<td>26.2 (20.3-35.2)</td>
<td>26.1 (20.1-35.4)</td>
<td>25.0 (19.9-33.1)</td>
<td>25.2 (20.1-33.0)</td>
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<td>390 (15.8%)</td>
<td>696 (15.6%)</td>
<td>256 (5.3%)</td>
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<td>Prothrombin G20210A, n (%)</td>
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<td>231 (5.2%)</td>
<td>94 (1.9%)</td>
<td>52 (1.8%)</td>
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<td>Arterial thrombosis, n (%)</td>
<td>147 (6.7%)</td>
<td>309 (6.9%)</td>
<td>264 (4.6%)</td>
<td>133 (4.8%)</td>
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<td>Malignancy, n (%)</td>
<td>201 (8.2%)</td>
<td>696 (13.5%)</td>
<td>236 (3.8%)</td>
<td>107 (3.7%)</td>
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<td>Diabetes mellitus, n (%)</td>
<td>85 (3.9%)</td>
<td>190 (4.3%)</td>
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<td>269 (5.2%)</td>
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<td>Immobilization, * n (%)</td>
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<td>1670 (35.1%)</td>
<td>923 (14.8%)</td>
<td>421 (14.4%)</td>
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<td>Surgery, † n (%)</td>
<td>542 (21.9%)</td>
<td>1155 (22.9%)</td>
<td>418 (6.7%)</td>
<td>202 (6.9%)</td>
</tr>
</tbody>
</table>

*Immobilization defined as bedridden for more than 4 days or hospitalization within 1 year prior to the index date. †Surgery within 1 year prior to the index date.
<table>
<thead>
<tr>
<th>Hemostatic factor</th>
<th>Adjusted (^1) mean difference (95% confidence interval) as compared with &gt;50th percentile (eGFR &gt; 86 ml/min) N=1468</th>
<th>eGFR continuous scale</th>
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<tr>
<td></td>
<td>10(^{th}) - 50(^{th}) percentile (eGFR 68-86 ml/min) N=1175</td>
<td>1(^{st}) - 2.5(^{th}) percentile (eGFR 53-64 ml/min) N=43</td>
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<tr>
<td><strong>Anticoagulant factors</strong></td>
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<tr>
<td>Protein S*, IU/dL</td>
<td>0 (-1 to 2)</td>
<td>3 (-2 to 7)</td>
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<tr>
<td>Protein C*, IU/dL</td>
<td>0 (-2 to 2)</td>
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</tr>
<tr>
<td>Antithrombin, IU/dL</td>
<td>0 (-1 to 1)</td>
<td>1 (-2 to 4)</td>
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<td><strong>Procoagulant factors</strong></td>
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<td>-0.1 (-0.1 to 0.0)</td>
<td>0 (-0.2 to 0.1)</td>
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<td>Factor II*, IU/dL</td>
<td>1 (0 to 2)</td>
<td>4 (1 to 6)</td>
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<tr>
<td>Factor VII*, IU/dL</td>
<td>1 (-1 to 3)</td>
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<td>Factor VIII, IU/dL</td>
<td>5 (1 to 8)</td>
<td>17 (10 to 24)</td>
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<td>12 (5 to 19)</td>
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<td>Factor IX*, IU/dL</td>
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<td>1 (-2 to 4)</td>
</tr>
<tr>
<td>Factor X*, IU/dL</td>
<td>0 (-1 to 2)</td>
<td>2 (-1 to 5)</td>
</tr>
<tr>
<td>Factor XI, IU/dL</td>
<td>2 (0 to 3)</td>
<td>1 (-2 to 5)</td>
</tr>
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\(\)Adjusted for age and sex. Vitamin-K users excluded from the analyses.

Table 2. Effect of percentiles of kidney function on hemostatic factors in control subjects.
Table 3. Effect of mildly and moderately to severely decreased kidney function on hemostatic factors in control subjects

<table>
<thead>
<tr>
<th>Hemostatic factor</th>
<th>Adjusted* mean difference (95% confidence interval) as compared with normal kidney function (eGFR &gt;90 ml/min)</th>
<th>Pooled controls</th>
<th>Partner controls</th>
<th>RDD controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>eGFR 60-90 ml/min</td>
<td>eGFR &lt;60 ml/min</td>
<td>eGFR 60-90 ml/min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N=1672</td>
<td>N=85</td>
<td>N=836</td>
</tr>
<tr>
<td><strong>Anticoagulant factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein S*, IU/dL</td>
<td>0 (-1 to 2)</td>
<td>4 (0 to 9)</td>
<td>0 (-3 to 2)</td>
<td>3 (-4 to 10)</td>
</tr>
<tr>
<td>Protein C*, IU/dL</td>
<td>0 (-2 to 1)</td>
<td>0 (-5 to 5)</td>
<td>-2 (-4 to 0)</td>
<td>-5 (-12 to 2)</td>
</tr>
<tr>
<td>Antithrombin, IU/dL</td>
<td>0 (-1 to 1)</td>
<td>1 (-1 to 4)</td>
<td>-1 (-2 to 1)</td>
<td>2 (-1 to 6)</td>
</tr>
<tr>
<td><strong>Procoagulant factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>-0.1 (-0.1 to 0.0)</td>
<td>0.3 (0.1 to 0.4)</td>
<td>-0.1 (-0.1 to 0.0)</td>
<td>0.3 (0.1 to 0.5)</td>
</tr>
<tr>
<td>Factor II*, IU/dL</td>
<td>1 (-1 to 2)</td>
<td>0 (-4 to 4)</td>
<td>0 (-2 to 1)</td>
<td>-3 (-9 to 4)</td>
</tr>
<tr>
<td>Factor VII*, IU/dL</td>
<td>1 (-1 to 3)</td>
<td>10 (5 to 16)</td>
<td>1 (-2 to 3)</td>
<td>6 (-2 to 14)</td>
</tr>
<tr>
<td>Factor VIII, IU/dL</td>
<td>6 (2 to 9)</td>
<td>41 (31 to 51)</td>
<td>6 (1 to 11)</td>
<td>39 (24 to 54)</td>
</tr>
<tr>
<td>VWF, IU/dL</td>
<td>3 (-1 to 6)</td>
<td>32 (21 to 43)</td>
<td>3 (-2 to 8)</td>
<td>35 (19 to 52)</td>
</tr>
<tr>
<td>Factor IX*, IU/dL</td>
<td>-1 (-3 to 1)</td>
<td>3 (-1 to 8)</td>
<td>-2 (-4 to 0)</td>
<td>0 (-7 to 6)</td>
</tr>
<tr>
<td>Factor X*, IU/dL</td>
<td>0 (-2 to 1)</td>
<td>1 (-4 to 5)</td>
<td>-1 (-4 to 4)</td>
<td>-3 (-9 to 4)</td>
</tr>
<tr>
<td>Factor XI, IU/dL</td>
<td>2 (0 to 3)</td>
<td>7 (3 to 11)</td>
<td>1 (-1 to 3)</td>
<td>11 (5 to 18)</td>
</tr>
</tbody>
</table>

*Vitamin-K users excluded from the analyses. †Adjusted for age and sex.
Table 4. Kidney function and risk of venous thrombosis

<table>
<thead>
<tr>
<th>Percentiles</th>
<th>eGFR (ml/min)</th>
<th>Thrombosis</th>
<th>Controls</th>
<th>Adjusted for age and sex</th>
<th>+ body mass index, diabetes mellitus, malignancy, arterial thrombosis, prothrombin G20210A, and factor V Leiden</th>
<th>+ immobilization, surgery, and corticosteroid use</th>
<th>+ von willebrand factor, and factor VIII</th>
<th>+ factor II, fibrinogen, factor IX, factor X, factor XI, protein S, protein C, and antithrombin</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;50th</td>
<td>&gt;86</td>
<td>1165</td>
<td>1468</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>10th - 50th</td>
<td>68-86</td>
<td>943</td>
<td>1175</td>
<td>1.1 (0.9-1.2)</td>
<td>1.1 (1.0-1.3)</td>
<td>1.1 (1.0-1.3)</td>
<td>1.0 (0.9-1.2)</td>
<td>1.1 (0.9-1.1)</td>
</tr>
<tr>
<td>5th - 10th</td>
<td>64-68</td>
<td>132</td>
<td>147</td>
<td>1.2 (1.0-1.6)</td>
<td>1.2 (0.9-1.6)</td>
<td>1.2 (0.9-1.6)</td>
<td>1.0 (0.7-1.3)</td>
<td>1.0 (0.7-1.3)</td>
</tr>
<tr>
<td>2.5th - 5th</td>
<td>59-64</td>
<td>96</td>
<td>74</td>
<td>1.8 (1.3-2.5)</td>
<td>1.9 (1.4-2.7)</td>
<td>1.9 (1.3-2.7)</td>
<td>1.4 (1.0-2.1)</td>
<td>1.4 (0.9-2.1)</td>
</tr>
<tr>
<td>1st - 2.5th</td>
<td>53-64</td>
<td>62</td>
<td>43</td>
<td>2.0 (1.4-3.1)</td>
<td>2.0 (1.3-3.1)</td>
<td>1.8 (1.2-2.9)</td>
<td>1.3 (0.8-2.2)</td>
<td>1.3 (0.8-2.2)</td>
</tr>
<tr>
<td>&lt;1st</td>
<td>&lt;53</td>
<td>75</td>
<td>29</td>
<td>3.7 (2.4-5.7)</td>
<td>2.8 (1.7-4.6)</td>
<td>2.4 (1.5-4.1)</td>
<td>1.0 (0.5-1.8)</td>
<td>0.8 (0.4-1.5)</td>
</tr>
</tbody>
</table>
Table 5. Effect of mildly and moderately to severely decreased kidney function on venous thrombosis

<table>
<thead>
<tr>
<th>eGFR (ml/min)</th>
<th>Thrombosis</th>
<th>Pooled controls</th>
<th>ODDS RATIOS (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>&gt;90</td>
<td>937 (37.9)</td>
<td>1179 (40.2)</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>60-90</td>
<td>1376 (55.6)</td>
<td>1672 (56.9)</td>
<td>1.1 (1.0-1.2)</td>
</tr>
<tr>
<td>&lt;60</td>
<td>160 (6.5)</td>
<td>85 (2.9)</td>
<td>2.6 (2.0-3.5)</td>
</tr>
<tr>
<td>Thrombosis</td>
<td>Patients</td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>&gt;90</td>
<td>467 (35.5)</td>
<td>538 (40.9)</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>60-90</td>
<td>756 (57.4)</td>
<td>745 (56.6)</td>
<td>1.3 (1.1-1.5)</td>
</tr>
<tr>
<td>&lt;60</td>
<td>93 (7.1)</td>
<td>33 (2.5)</td>
<td>3.8 (2.4-6.0)</td>
</tr>
<tr>
<td>Partner controls</td>
<td>N (%)</td>
<td>N (%)</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>&gt;90</td>
<td>937 (37.9)</td>
<td>576 (39.6)</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>60-90</td>
<td>1376 (55.6)</td>
<td>836 (57.4)</td>
<td>1.0 (0.9-1.2)</td>
</tr>
<tr>
<td>&lt;60</td>
<td>160 (6.5)</td>
<td>44 (3.0)</td>
<td>2.2 (1.6-3.2)</td>
</tr>
</tbody>
</table>
Table 6. Effect of mildly and moderately to severely decreased kidney function on venous thrombosis (CKD-EPI equation)

<table>
<thead>
<tr>
<th>eGFR (ml/min)</th>
<th>Thrombosis</th>
<th>Controls</th>
<th>Adjusted for age and sex</th>
<th>ODDS RATIOS (95% CI)</th>
<th>+ body mass index, diabetes mellitus, malignancy, arterial thrombosis, prothrombin G20210A, and factor V Leiden</th>
<th>+ immobilization, surgery, and corticosteroid use</th>
<th>+ von willebrand factor, and factor VIII</th>
<th>+ factor II, fibrinogen, factor IX, factor X, factor XI, protein S, protein C, and antithrombin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
<td>1 (reference)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>&gt;90</td>
<td>1496 (60.5)</td>
<td>1856 (63.2)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>60-90</td>
<td>865 (35.0)</td>
<td>1031 (35.1)</td>
<td>1.1 (1.0-1.3)</td>
<td>1.2 (1.0-1.3)</td>
<td>1.1 (1.0-1.3)</td>
<td>1.0 (0.9-1.2)</td>
<td>1.0 (0.9-1.2)</td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>112 (4.5)</td>
<td>49 (1.7)</td>
<td>3.3 (2.3-4.6)</td>
<td>2.7 (1.8-4.0)</td>
<td>2.5 (1.6-3.7)</td>
<td>1.3 (0.8-2.0)</td>
<td>1.1 (0.7-1.8)</td>
<td></td>
</tr>
</tbody>
</table>
Figure Legends:

**Figure 1.** Flow chart MEGA study.

**Figure 2.** Causal diagram on the association between kidney function and venous thrombosis.

**Figure 3.** Percentiles of kidney function and risk of venous thrombosis.
*Hemostatic factors could be both confounders and mediators in the association between kidney function and venous thrombosis.
Role of Hemostatic Factors on the Risk of Venous Thrombosis in Persons with Impaired Kidney Function

Gürbey Ocak, Carla Y. Vossen, Willem M. Lijfering, Marion Verduijn, Friedo W. Dekker, Frits R. Rosendaal and Suzanne C. Cannegieter

Circulation. published online November 8, 2013;

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### SUPPLEMENTAL MATERIAL

**Supplemental Table 1. Baseline characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Venous thrombosis</th>
<th>Partner controls</th>
<th>RDD controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=2473</td>
<td>N=1480</td>
<td>N=1456</td>
</tr>
<tr>
<td>Median age, years (5-95th %)</td>
<td>49.1 (25.9-67.5)</td>
<td>51.0 (28.6-66.4)</td>
<td>48.4 (24.7-67.5)</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>1346 (54.4%)</td>
<td>760 (51.4%)</td>
<td>783 (53.8%)</td>
</tr>
<tr>
<td>BMI, kg/m² (5-95th %)</td>
<td>26.2 (20.3-35.2)</td>
<td>25.7 (20.4-33.3)</td>
<td>24.7 (19.7-32.5)</td>
</tr>
<tr>
<td>Factor V Leiden, n (%)</td>
<td>390 (15.8%)</td>
<td>68 (4.6%)</td>
<td>77 (5.3%)</td>
</tr>
<tr>
<td>Prothrombin G20210A, n (%)</td>
<td>117 (4.7%)</td>
<td>30 (2.0%)</td>
<td>22 (1.5%)</td>
</tr>
<tr>
<td>Arterial thrombosis, n (%)</td>
<td>147 (6.7%)</td>
<td>69 (5.1%)</td>
<td>64 (4.4%)</td>
</tr>
<tr>
<td>Malignancy, n (%)</td>
<td>201 (8.2%)</td>
<td>41 (2.8%)</td>
<td>66 (4.5%)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>85 (3.9%)</td>
<td>47 (3.5%)</td>
<td>42 (2.9%)</td>
</tr>
<tr>
<td>Corticosteroid use, n (%)</td>
<td>113 (4.8%)</td>
<td>13 (0.9%)</td>
<td>16 (1.1%)</td>
</tr>
<tr>
<td>Immobilization,* n (%)</td>
<td>774 (31.5%)</td>
<td>188 (12.8%)</td>
<td>233 (16.1%)</td>
</tr>
<tr>
<td>Surgery,† n (%)</td>
<td>542 (21.9%)</td>
<td>85 (5.8%)</td>
<td>117 (8.1%)</td>
</tr>
</tbody>
</table>

*Immobilization defined as bedridden for more than 4 days or hospitalization within 1 year prior to the index date. †Surgery within 1 year prior to the index date