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Chapter 4

Role of hemostatic factors on the risk of venous thrombosis in persons with impaired kidney function

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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: 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).

Results: Several hemostatic factors showed a procoagulant shift with decreasing kidney function in controls, most notably factor VIII and von Willebrand factor (VWF). 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.

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

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 and 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), that in their turn increase the risk of venous thrombosis such as immobilization,⁹ surgery,⁹ corticosteroid use,¹⁴ or changes in hemostatic factors.⁹

Therefore, the aim of our study was 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 (Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis study [MEGA study]).

METHODS

Study design

MEGA is a large, population-based case-control study of 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.¹⁵ This study was approved by the Ethics Committee of the Leiden University Medical Center and written informed consent was obtained from all 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.¹⁵ 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 an 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, that is, of 164 partners, the corresponding patient originally participated, but was later found not to be eligible (age >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.

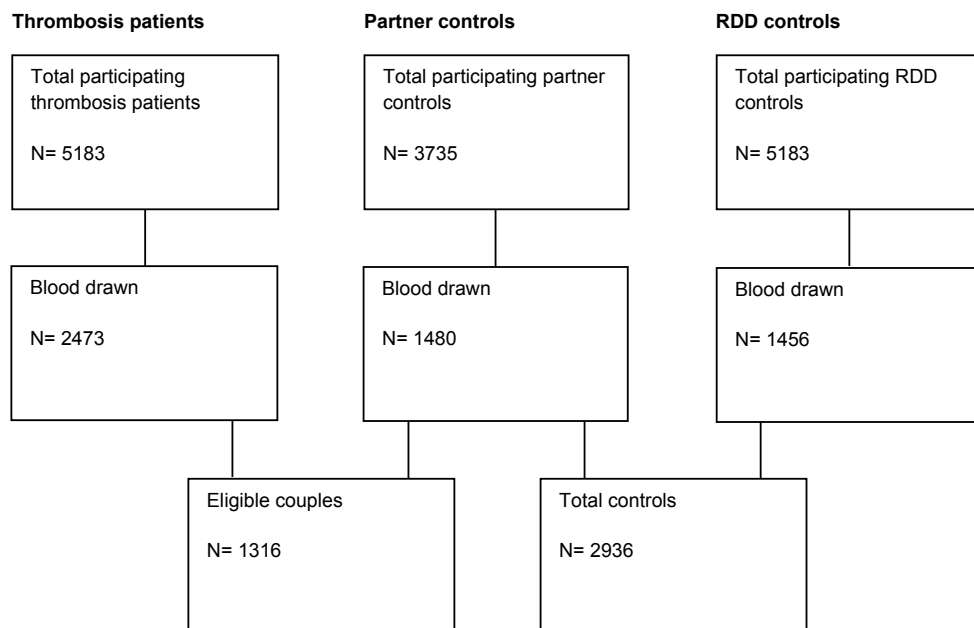


Figure 1. Flow chart of the MEGA study

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, 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 completing 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.¹⁶ The common genetic risk factors factor V Leiden and prothrombin G20210A were determined with the TaqMan assay.¹⁷ 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 for using these 6 percentile groups was to investigate a wide range of eGFR values, 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. We subsequently included factors that might mediate the increased risk of VT associated with chronic kidney disease, that is, immobilization, surgery, and corticosteroid use. We also adjusted for hemostatic factors (as continuous variables) (Figure 2). Lastly, we reanalyzed the data using clinical cutoff 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 Chronic Kidney Disease Epidemiology Collaboration (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 with the pooled control groups, (2) patients compared with partner controls (conditional logistic regression), and (3) patients compared with RDD controls (unconditional logistic regression). The first analysis used nonconservative estimates of the standard errors, whereas the second and third analyses provide overly conservative estimates when applied to the pooled analysis.

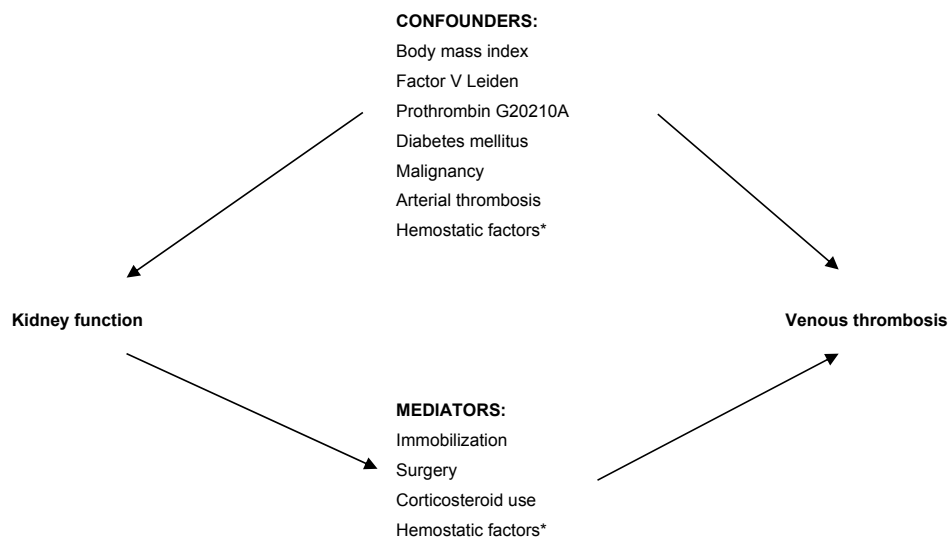


Figure 2. Causal diagram on the association between kidney function and venous thrombosis

*Hemostatic factors could be both confounders and mediators in the association between kidney function and venous thrombosis

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 to 6 months, 6 to 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 subjects, 2936 provided blood samples. There were no substantial differences in the baseline characteristics in all participants compared with participants who 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 patients with thrombosis than in controls. Furthermore, compared with controls, patients with venous thrombosis 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.

Hemostatic factor levels in controls in relation to kidney function

In controls, several hemostatic factors showed a shift towards a procoagulant state with decreasing kidney function (Table 2). Compared with 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 in factor VIII (60 IU/dL; 95%CI 44 to 76) and von Willebrand factor (60 IU/dL; 95%CI 43 to 77). A 10 ml/min decrease in eGFR was associated with 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.

Table 1. Baseline characteristics

	Patients in MEGA N=5183	Patients tested N=2473	Controls in MEGA N=6297	Controls tested N=2936
Median age, years (5-95th %)	49.1 (25.9-67.5)	49.6 (25.5-67.8)	47.7 (25.4-66.6)	49.8 (27.1-67.0)
Women, n (%)	1346 (54.4%)	2801 (54.0%)	3383 (53.7%)	1543 (52.6%)
BMI, kg/m ² (5-95th %)	26.2 (20.3-35.2)	26.1 (20.1-35.4)	25.0 (19.9-33.1)	25.2 (20.1-33.0)
Factor V Leiden, n (%)	390 (15.8%)	696 (15.6%)	256 (5.3%)	145 (4.9%)
Prothrombin G20210A, n (%)	117 (4.7%)	231 (5.2%)	94 (1.9%)	52 (1.8%)
Arterial thrombosis, n (%)	147 (6.7%)	309 (6.9%)	264 (4.6%)	133 (4.8%)
Malignancy, n (%)	201 (8.2%)	696 (13.5%)	236 (3.8%)	107 (3.7%)
Diabetes mellitus, n (%)	85 (3.9%)	190 (4.3%)	196 (3.4%)	89 (3.2%)
Corticosteroid use, n (%)	113 (4.8%)	269 (5.2%)	64 (1.0%)	29 (1.0%)
Immobilization,* n (%)	774 (31.5%)	1670 (35.1%)	923 (14.8%)	421 (14.4%)
Surgery,† n (%)	542 (21.9%)	1155 (22.9%)	418 (6.7%)	202 (6.9%)

*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 2. Effect of percentiles of kidney function on hemostatic factors in control subjects*

Hemostatic factor	Adjusted† mean difference (95% confidence interval) as compared with >50th percentile (eGFR >86 ml/min) N=1468					eGFR continuous scale
	10 th - 50 th percentile (eGFR 68-86 ml/min) N=1175	5 th - 10 th percentile (eGFR 64-68 ml/min) N=147	2.5 th - 5 th percentile (eGFR 59-64 ml/min) N=74	1 st - 2.5 th percentile (eGFR 53-64 ml/min) N=43	<1 st percentile (eGFR <53 ml/min) N=29	eGFR per 10 ml/min increase N=2936
Anticoagulant factors						
Protein S*, IU/dL	0 (-1 to 2)	3 (0 to 7)	3 (-2 to 7)	3 (-3 to 9)	4 (-11 to 19)	-0.4 (-0.9 to 0.0)
Protein C*, IU/dL	0 (-2 to 2)	5 (1 to 8)	-1 (-6 to 4)	-1 (-8 to 5)	2 (-6 to 10)	0.1 (-0.4 to 0.5)
Antithrombin, IU/dL	0 (-1 to 1)	1 (-1 to 3)	1 (-2 to 4)	2 (-2 to 5)	2 (-2 to 6)	0.0 (-0.2 to 0.3)
Procoagulant factors						
Fibrinogen, g/L	-0.1 (-0.1 to 0.0)	-0.1 (-0.2 to 0.1)	0.0 (-0.2 to 0.1)	0.0 (-0.2 to 0.2)	0.7 (0.5 to 0.9)	0.003 (-0.01 to 0.02)
Factor II*, IU/dL	1 (0 to 2)	4 (1 to 6)	-1 (-5 to 3)	-2 (-7 to 3)	3 (-3 to 9)	-0.3 (-0.6 to 0.1)
Factor VII*, IU/dL	1 (-1 to 3)	7 (3 to 11)	1 (-5 to 7)	2 (-5 to 9)	31 (22 to 40)	-1.0 (-1.6 to -0.5)
Factor VIII, IU/dL	5 (1 to 8)	17 (10 to 24)	20 (10 to 30)	26 (13 to 39)	60 (44 to 76)	-2.8 (-3.8 to -1.7)
VWF, IU/dL	1 (-2 to 5)	12 (5 to 19)	19 (9 to 30)	12 (-1 to 25)	60 (43 to 77)	-1.9 (-2.9 to -0.8)
Factor IX*, IU/dL	-1 (-2 to 1)	1 (-2 to 4)	-2 (-6 to 3)	1 (-5 to 7)	12 (5 to 19)	0.1 (-0.3 to 0.6)
Factor X*, IU/dL	0 (-1 to 2)	2 (-1 to 5)	0 (-5 to 4)	2 (-4 to 8)	4 (-4 to 11)	-0.2 (-0.7 to 0.2)
Factor XI, IU/dL	2 (0 to 3)	1 (-2 to 5)	2 (-3 to 6)	6 (0 to 11)	10 (3 to 17)	-0.7 (-1.1 to -0.3)

*Vitamin-K users were excluded from the analyses. †Adjusted for age and sex

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 compared with subjects with normal kidney function (eGFR >90 ml/min), 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) (Table 3). These results were in the same range for partner controls and for RDD controls. factor VIII (41 IU/dL; 95% CI 31-51) and von Willebrand factor (32 IU/dL; 95% CI 21-43) (Table 3). 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. Compared with subjects in the >50th percentile, decreasing eGFR was associated with a steadily increasing risk, that is, 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 the 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 compared with participants with levels <100 IU/dL. Results were in the same direction and risks were similarly attenuated after adjustment for the coagulation factors when both control groups were analyzed separately.

Table 4. Kidney function and risk of venous thrombosis

Percentiles		eGFR (ml/min)	Thrombosis Patients	Controls	Adjusted for age and sex	ODDS RATIOS (95% CI)				
			N	N		+ body mass index, diabetes mellitus, malignancy, arterial thrombosis, prothrombin G20210A, and factor V Leiden	+ immobilization, surgery, and corticosteroid use	+ von willebrand factor, and factor VIII	+ factor II, fibrinogen, factor IX, factor X, factor XI, protein S, protein C, and antithrombin	
>50 th		>86	1165	1468	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	
10 th - 50 th		68-86	943	1175	1.1 (0.9-1.2)	1.1 (1.0-1.3)	1.1 (1.0-1.3)	1.0 (0.9-1.2)	1.1 (0.9-1.1)	
5 th - 10 th		64-68	132	147	1.2 (1.0-1.6)	1.2 (0.9-1.6)	1.2 (0.9-1.6)	1.0 (0.7-1.3)	1.0 (0.7-1.3)	
2.5 th - 5 th		59-64	96	74	1.8 (1.3-2.5)	1.9 (1.4-2.7)	1.9 (1.3-2.7)	1.4 (1.0-2.1)	1.4 (0.9-2.1)	
1 st - 2.5 th		53-64	62	43	2.0 (1.4-3.1)	2.0 (1.3-3.1)	1.8 (1.2-2.9)	1.3 (0.8-2.2)	1.3 (0.8-2.2)	
<1 st		<53	75	29	3.7 (2.4-5.7)	2.8 (1.7-4.6)	2.4 (1.5-4.1)	1.0 (0.5-1.8)	0.8 (0.4-1.5)	

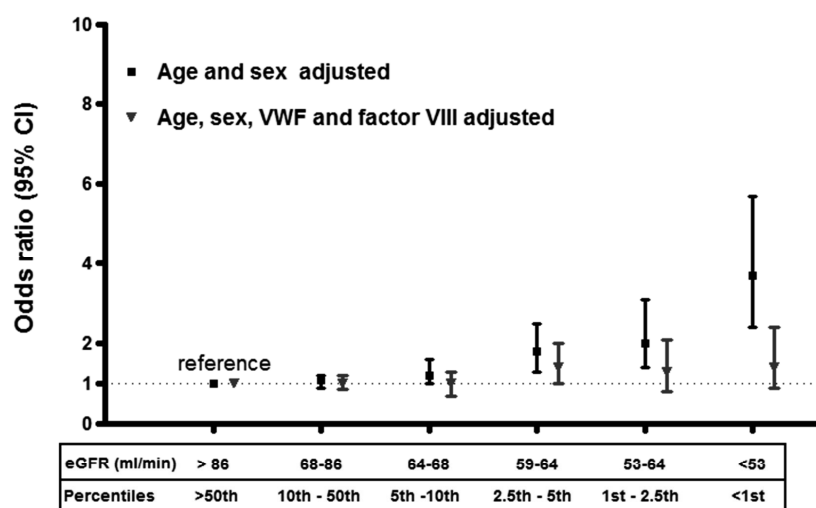


Figure 3. Percentiles of kidney function and risk of venous thrombosis

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 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 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). In both the partner controls and RDD controls, odds ratios were 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, because the MDRD equation may underestimate glomerular filtration rates at borderline abnormal levels (i.e., 60mL/minute), although 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.

Table 5. Effect of mildly and moderately to severely decreased kidney function on venous thrombosis

		ODDS RATIOS (95% CI)					
eGFR (ml/min)	Thrombosis Patients	Pooled controls	Adjusted for age and sex	+ body mass index, diabetes mellitus, malignancy, arterial thrombosis, prothrombin G20210A, and factor V Leiden	+ immobilization, surgery, and corticosteroid use	+ von willebrand factor, and factor VIII	+ factor II, fibrinogen, factor IX, factor X, factor XI, protein S, protein C, and antithrombin
	N (%)	N (%)					
>90	937 (37.9)	1179 (40.2)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
60- 90	1376 (55.6)	1672 (56.9)	1.1 (1.0-1.2)	1.1 (1.0-1.3)	1.2 (1.0-1.4)	1.1 (0.9-1.2)	1.1 (0.9-1.3)
<60	160 (6.5)	85 (2.9)	2.6 (2.0-3.5)	2.3 (1.7-3.1)	2.2 (1.5-3.0)	1.2 (0.8-1.7)	1.1 (0.7-1.6)
Partner controls							
>90	467 (35.5)	538 (40.9)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
60- 90	756 (57.4)	745 (56.6)	1.3 (1.1-1.5)	1.3 (1.0-1.6)	1.3 (1.1-1.7)	1.2 (0.9-1.6)	1.2 (0.9-1.6)
<60	93 (7.1)	33 (2.5)	3.8 (2.4-6.0)	3.0 (1.7-5.1)	2.8 (1.6-4.9)	1.9 (1.0-3.7)	1.7 (0.9-3.2)
RDD controls							
>90	937 (37.9)	576 (39.6)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
60- 90	1376 (55.6)	836 (57.4)	1.0 (0.9-1.2)	1.1 (0.9-1.2)	1.1 (0.9-1.3)	1.0 (0.8-1.2)	1.0 (0.8-1.2)
<60	160 (6.5)	44 (3.0)	2.2 (1.6-3.2)	2.0 (1.4-2.9)	1.9 (1.3-2.8)	1.0 (0.7-1.6)	0.9 (0.6-1.5)

Table 6. Effect of mildly and moderately to severely decreased kidney function on venous thrombosis (CKD-EPI equation)

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

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 to 6 months (mean 87 ml/min), 6 to 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, 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.

In both the Longitudinal Investigation of Thromboembolism Etiology (LITE)⁴ and Prevention of Renal and Vascular End-Stage Disease (PREVEND)⁵ study, chronic kidney disease was associated with an increased risk of venous thrombosis. However, in neither study were factors that might explain the association between chronic kidney disease and venous thrombosis identified. Our analyses showed that the presence of common risk factors for chronic kidney disease and venous thrombosis, such as 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} 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 in turn increase the risk of venous thrombosis (mediators), such as immobilization,⁹ surgery,⁹ corticosteroid use,¹⁴ and changes in hemostatic factors.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 >3 grams per 24 hours) were shown to have elevated levels of fibrinogen, factor VIII and von Willebrand factor.²¹⁻²³ In addition, patients with nephrotic syndrome have decreased antithrombin levels as a result of urinary loss of antithrombin.²⁴ Increased levels of fibrinogen,^{25,26} factor VIII,²⁷ factor IX,⁹ factor XI,⁹ and von Willebrand factor²⁸ 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.^{27,29} In our study, we observed a procoagulant shift in subjects with an impaired kidney function <1st percentile corresponding to 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, estimated with either the MDRD equation or 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. Because von Willebrand factor and factor VIII are markers of endothelial damage,³⁰ 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 and 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). A drawback of cohort studies is 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. Because 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 to 6 months, 6 to 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, because 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 patients with venous thrombosis and 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.³¹ 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, because 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. Additionally, 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 >3 grams per 24 hours), has been associated with venous thrombosis.²³⁻²⁵ 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. Our aim was 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. The reason is that most of the subjects with impaired kidney function in our study had no symptoms and were never, or had not yet been diagnosed with impaired kidney function. It would certainly be useful to study the risks of thrombosis for the various types of primary kidney disease. Rather than comparing patients with thrombosis with controls, patients with specific kidney disorders should be followed for the development of thrombosis, because these various diseases are 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 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.

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