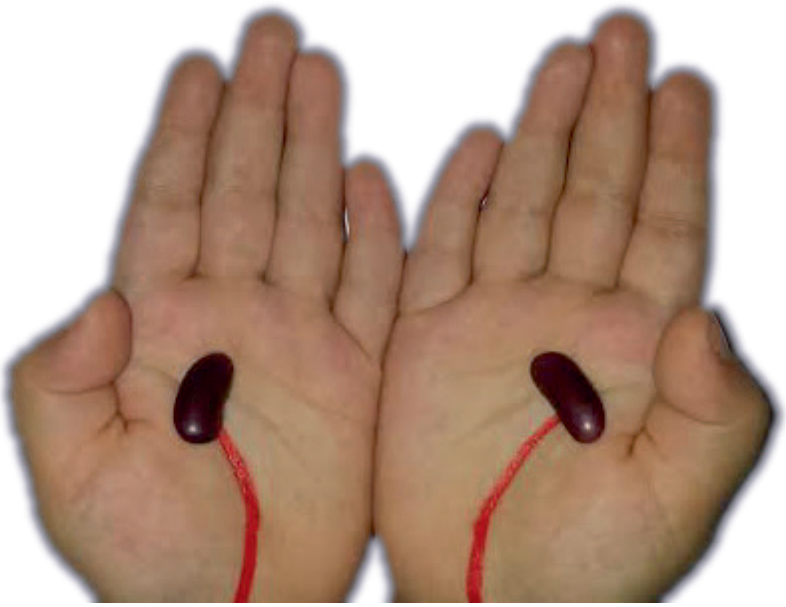
VASCULAR COMPLICATIONS IN KIDNEY DISEASE



Gürbey Ocak

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Voor mijn ouders, Meriem en Safiye

CONTENTS

Chapter 1

General introduction

1. BACKGROUND

Chronic kidney disease is an important public health problem worldwide. The prevalence of chronic kidney disease has been estimated around 13% in the United States and around 10% in the Netherlands.[1,2] A progressive loss in over a period of months or years leading to inadequate removal of fluid and waste products is characteristic for chronic kidney disease. The diagnosis of chronic kidney disease is often made in patients who are known to be at risk of kidney problems, such as those with hypertension or diabetes mellitus.[3]

Chronic kidney disease is defined as persistent renal damage (detected by a renal biopsy or albuminuria) or a glomerular filtration rate below the 60 ml/min for three months or longer.[4] The diagnosis of chronic kidney disease is irrespective of the underlying type of kidney disease and can be classified into five clinical stages according to the guidelines of the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) (Table 1).[4] Patients with stage 5 chronic kidney disease are often considered for renal replacement therapy in the form of dialysis (hemodialysis or peritoneal dialysis) or transplantation. For hemodialysis, vascular access, i.e., a catheter, fistula or graft, is needed for dialysis. For peritoneal dialysis, a catheter is implanted in the abdominal cavity of the patient.

Table 1. Classification of chronic kidney disease

Stage	Glomerular filtration rate (ml/min)	Kidney damage Albuminuria (>30 mg/24h)
1	>90	Yes
2	60-90	Yes
3	30-60	Not necessary for diagnosis
4	15-30	Not necessary for diagnosis
5	<15	Not necessary for diagnosis

1.1 Vascular complications in patients with kidney disease

Vascular complications such as arterial thrombosis (myocardial infarction or ischemic stroke), venous thrombosis (deep vein thrombosis or pulmonary embolism) and vascular access (fistula, graft or catheter) related complications in patients on hemodialysis are important threats for chronic kidney disease patients. Many hospitalizations and deaths in chronic kidney disease patients, especially in dialysis patients, are consequences of vascular related problems.[5-11]

1.1.1 Venous and arterial thrombosis

Venous thrombosis is a collective term for deep vein thrombosis and pulmonary embolism, and several more rare forms of obstructive clots in veins. Established risk factors for venous

thrombosis include immobilization, surgery, pregnancy, malignancy, hormone replacement therapy, oral contraceptive use and genetic risk factors (including factor V Leiden).[12] Arterial thrombosis, including coronary heart disease and ischemic stroke, is mostly secondary to atherosclerosis due to hypertension, hyperlipidemia, diabetes, smoking and obesity.[13] Chronic kidney disease is an established risk factor for arterial thrombosis.[14-16] Until recently, it has been unclear whether chronic kidney disease also increases the risk of venous thrombosis. The Longitudinal Investigation of Thromboembolism Etiology (LITE) study was the first study that showed an increased risk of venous thrombosis for patients with a chronic kidney disease in individuals older than 45 years of age.[17]

The LITE study focused on patients with stage 3 chronic kidney. However, studies that investigated the risk of venous thrombosis in the early stages of chronic kidney disease were lacking as well as studies that investigate the risk of venous and arterial thrombosis in dialysis patients. Furthermore, the association between kidney disease and venous thrombosis was not explained and no studies were available that investigated high-risk groups of patients with chronic kidney disease that may benefit from thromboprophylaxis.

1.1.2 Vascular accesses in patient on hemodialysis

Hemodialysis patients require vascular accesses for hemodialysis therapy. The options to gain vascular access are fistulas, grafts or catheters. While evidence from randomized-controlled trials is lacking, there is a broad consensus that fistulas and grafts are superior to central venous catheters. Catheter use for hemodialysis has been associated with increased risk of thrombotic complications,[18,19] short access survival,[19,20] and increased risk of infections.[21-24] Therefore, the National Kidney Foundation Kidney Disease Outcome Quality Initiative (NKF K/DOQI) guidelines[25] recommend the use of fistulas or grafts instead of catheters for vascular access in hemodialysis patients.

Despite this preference and recommendation for graft or fistula use instead of catheters, only few studies have investigated the association between catheter use and mortality in hemodialysis patients.[27-29] Furthermore, a limited number of studies have investigated the association between fistula use versus graft use and mortality.[29,30]

1.2 Objective and outline of this thesis

The main objective of this thesis was to investigate vascular complications in patients with kidney disease. Specifically, our aims were to:

- investigate the association between kidney disease and venous and arterial thrombosis
- provide insight in the mechanism of the association between kidney disease and thrombosis

- investigate the mortality risks for hemodialysis patients with catheter, fistula or graft vascular accesses
- investigate the association between genetic risk factors for arterial and venous thrombosis and mortality in dialysis patients

These results may help to define new treatment strategies in the prevention of venous thrombosis and in the treatment of hemodialysis patients.

1.3 Clinical data used in this thesis

In this thesis, data from the **MEGA** (Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis) study, the **PREVEND** (Prevention of Renal and Vascular Disease) study, the **NECOSAD** (Netherlands Cooperative Study on the Adequacy of Dialysis) study, the **ERA-EDTA** (European Renal Association-European Dialysis and Transplant Association) Registry, and the **4D** (German Diabetes Dialysis) study were used.

1.3.1 MEGA study

The studies described in **chapter 2, 4, and 5** are based on data collected from the MEGA study. The MEGA 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. As control persons, initially partners of patients aged <70 years without venous thrombosis were included. Later on persons without venous thrombosis were approached via a random-digit-dialing method.

In **chapter 2**, we investigated the risk of venous thrombosis for several self-reported major illnesses, including kidney disease, liver disease, rheumatoid arthritis, multiple sclerosis, heart failure, hemorrhagic stroke, and arterial thrombosis. In **chapter 4**, we investigated whether the association between kidney disease and venous thrombosis could be explained by body mass index, immobilization, surgery, corticosteroid use, diabetes mellitus, malignancy, arterial disorders, factor V Leiden, prothrombin G20210A, and coagulation factor levels. Based on our studies in chapter 2 and 3 in which we found an increased risk of venous thrombosis in kidney disease patients, we set out to identify kidney disease patients who were at high risk of venous thrombosis. Therefore, in **chapter 5**, we investigated joint effects of decreased kidney function with one or more other risk factors for thrombosis to identify high-risk groups that may benefit from thromboprophylaxis.

1.3.2 PREVEND study

For the study described in **chapter 3**, we collaborated with the Prevention of Renal and Vascular Disease (PREVEND) study group from the University Medical Center Groningen. The PREVEND study is a prospective cohort study in which all inhabitants of the city of Groningen, The Netherlands, aged 28–75 years (n = 85 421) were invited to send a morning urine sample to be screened for albuminuria. Of the responders, the PREVEND cohort was selected and tested for renal function and proteinuria in 24-hours urine samples. The study participants (n = 8 495) were followed for the occurrence of several outcomes, including venous thrombosis. In the PREVEND cohort, we determined the absolute and relative risks of venous thrombosis for patients with chronic kidney disease stages 1, 2, and 3.

1.3.3 NECOSAD study

For the studies described in **chapters 6, 8, 9 and 10**, we used data from the **NECOSAD** study. The NECOSAD is a prospective multicenter cohort study in which incident adult end-stage renal disease patients in the Netherlands were included from 38 dialysis centers. Outcome measures used in our study were patency loss, venous and arterial thrombosis, and death.

Chapters 2, 3 and 5 focused on the risk of venous thrombosis in kidney disease patients in earlier stages. In **chapter 6**, we explored the risk of venous and arterial thrombosis associated with dialysis. To that end, we assessed the absolute risk of deep vein thrombosis, pulmonary embolism, myocardial infarction and ischemic stroke in a cohort of end-stage renal disease patients receiving dialysis treatment.

In **chapter 8**, we investigated death risks in hemodialysis patients comparing catheter use with arteriovenous access use. In **chapter 9**, we compared patency loss and mortality between graft users and fistula users.

In the previous studies, we showed that dialysis patients were at increased risk of vascular access complications, and of venous and arterial thrombosis. Therefore, in **chapter 10**, we investigated whether polymorphisms in genes within the protein C pathway, which plays an important role in endothelial barrier function, anticoagulant processes and thrombosis, were associated with mortality in dialysis patients.

1.3.4 4D-study

For independent replication of the results of the NECOSAD study in **chapter 10**, we collaborated with the 4D-study group. The 4D-study was a double-blind, randomized trial on the effect of atorvastatin in hemodialysis patients with type 2 diabetes mellitus who had received less than two years of previous hemodialysis treatment. The primary endpoint was

a composite of cardiac death, non-fatal myocardial infarction and stroke, whichever occurred first. Patients were randomly assigned to either 20 mg of atorvastatin or placebo once daily until the date of death, censoring, or the end of study in March 2004.

1.3.5 ERA-EDTA Registry

For the study described in **chapter 7**, we collaborated with the ERA-EDTA Registry. The ERA-EDTA Registry collects data on renal replacement therapy, including date of birth, sex, primary kidney disease, date of start of renal replacement therapy, dialysis modality at baseline and during follow-up, and date and cause of death. We used a cohort of dialysis patients from 11 European countries: Austria, Belgium, Denmark, Finland, Greece, Iceland, Calabria (Italy), the Netherlands, Norway, Sweden and the autonomous communities of Andalusia, Asturias, Basque country, Catalonia, Castile-La Mancha, Castile and Leon, Extremadura, and Valencian region in Spain.

In **chapter 7**, we assessed the rates of death from myocardial infarction, stroke, and pulmonary embolism in an ERA EDTA cohort of incident dialysis patients and compared them with those in the general population.

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Chapter 2

Risk of venous thrombosis in patients with major illnesses: Results from the MEGA study

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ABSTRACT

Background: The risk of venous thrombosis associated with major illnesses is not well known, as is the combined effect of immobilization, and thrombophilia. The aim of this study was to assess the effect on the development of venous thrombosis of several major illnesses in combination with immobilization, BMI, and thrombophilia to identify high-risk groups that may provide a basis for personalized prevention.

Methods: This study included 4311 consecutive patients with a first episode of venous thrombosis and 5768 controls from a case-control study (MEGA study). We calculated odds ratios (ORs) for venous thrombosis for patients with a self-reported history of major illnesses.

Results: Venous thrombosis risk was increased for all investigated major illnesses: liver disease (OR) 1.7 (95%CI 1.0-2.9), kidney disease 3.7 (95%CI 2.3-5.9), rheumatoid arthritis 1.5 (95%CI 1.2-1.9), multiple sclerosis 2.4 (95%CI 1.3-4.3), heart failure 1.7 (95%CI 1.2-2.3), hemorrhagic stroke 4.9 (95%CI 2.4-9.9), arterial thrombosis 1.5 (95%CI 1.2-1.8), and in the presence of any of the above major illnesses 1.7 (95%CI 1.5-1.9). Combinations of major illnesses with immobilization and increased factor VIII (odds ratio 79.9; 95%CI 33.2-192.2), increased factor IX (35.3; 95%CI 14.2-87.8), increased von Willebrand factor (88.0; 95%CI 33.9-228.3), factor V Leiden (84.2; 95%CI 19.5-363.6), and blood group non-O (53.1; 95%CI 30.9-91.4) were associated with increased venous thrombosis risks.

Conclusion*:* All major illnesses reported here were associated with an increased risk of venous thrombosis. These risks were most pronounced at time of immobilization or in the presence of thrombophilia.

INTRODUCTION

Venous thrombosis occurs in 1-2 per 1000 persons annually.[1] Although venous thrombosis is a preventable disease, only a few provoking risk factors, such as surgery and hospitalization, are currently considered harmful enough to warrant prophylactic measures.[2]

A number of studies have reported on the risk of venous thrombosis in patients with a history of major illnesses including liver disease,[3-5] kidney disease,[6-9] rheumatoid arthritis,[4,10] multiple sclerosis,[11] heart failure,[4,12,13] hemorrhagic stroke,[14] and arterial thrombosis.[4,15-18] Some of these studies found a positive association between major illnesses and venous thrombosis,[4-15,18] whereas other studies did not.[3,16,17] The use of different definitions of major illnesses, together with variation in the studied populations, may explain this discrepancy. Studies on the association between kidney disease and venous thrombosis,[6-9] however, found consistent increased risks of venous thrombosis ranging from a 1.3-fold increased risk for patients with a mildly decreased kidney function[9] to an 8-fold in increased risk for patients with a nephrotic syndrome.[6] Even if these major illnesses were to be considered as risk factors for venous thrombosis, the risk may not be sufficiently high to justify the use of prophylaxis in all these situations, due to the increased risk of bleeding associated with most prophylactic measures (i.e. anticoagulant therapy).[19] The risk-benefit ratio may favor the use of such prophylactic measures only in persons at particularly increased risk of venous thrombosis. Patients with a major illness in combination with other risk factors, like immobilization or thrombophilia, who have high risks of venous thrombosis could benefit from prophylaxis during periods of high risks. Thus far, no studies have reported on the risk for venous thrombosis in persons with such combinations of prothrombotic conditions. Nor are there studies available that calculated the risk for venous thrombosis in patients with a major illness who are immobilized or have a genetic or acquired thrombophilia, such as factor (F) V Leiden or elevated levels of factor (F) VIII.

The aim of our study was to investigate the association between major illnesses (liver disease, kidney disease, rheumatoid arthritis, multiple sclerosis, heart failure, hemorrhagic stroke, and arterial thrombosis) and risk of venous thrombosis.

METHODS

Study design

The MEGA study (Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis study) is a large case-control study on risk factors for venous thrombosis, of which details have been published previously.[20,21] In brief, 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.

Patients

Only patients with a diagnosis of venous thrombosis that was confirmed with objective techniques were included in the analyses.[20,21] Exclusion criteria were severe psychiatric problems and inability to speak Dutch. Of the 6567 eligible patients, 5184 participated (79%). For the present analysis, questionnaire data on major illnesses were available from 4311 patients and 5768 controls, after exclusion of patients with a deep vein thrombosis of the arm (n=227).

Controls

As control persons, partners of patients aged <70 years without venous thrombosis were included, as well as persons without venous thrombosis obtained via a random-digit-dialing (RDD) method. Of the 5184 participating patients, 3735 had an eligible partner. Of the 3735 eligible partners, 2979 participated (80%) and completed a questionnaire including questions about the presence of major illnesses. The RDD control persons were recruited from the same geographical area as the patients, and were frequency matched to the patients on age and sex. Of the 4350 eligible random controls, four died before they were able to participate. Of the remaining 4346 individuals, 3000 participated (69%). Of the nonparticipants, 15 were in the end stage of disease and 1331 refused to participate or could not be located. A questionnaire was returned by 2789 of the participating random controls. This resulted in a total of 5768 control persons without venous thrombosis.

Data collection

All persons were asked to complete an extensive questionnaire on many potential risk factors for venous thrombosis. Of particular interest for this study question are items on general health characteristics (age, sex, body weight, height, and immobilization (defined as bedridden at home for at least 4 days, hospitalization, or surgery within three months prior to the index date)). Body mass index was calculated by dividing self-reported body weight (kg) by squared self-reported height (m^2). 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. The questionnaire also included questions about the presence of liver disease, kidney disease, rheumatoid arthritis, multiple sclerosis, heart failure, hemorrhagic stroke, and arterial thrombosis (myocardial infarction, angina, ischemic stroke, transient ischemic attack, and peripheral vascular disease) in the medical history.

Blood collection

Approximately 3 months after discontinuation of oral anticoagulant therapy, 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. All assays were performed by automated coagulation assays by laboratory technicians who were unaware of the case-control status of the samples. For logistic reasons, blood sampling was performed for patients up to June 2002; after this date only DNA was collected via buccal swabs. Plasma samples were available for 2134 of 4311 (50%) cases and 2812 of 5768 (49%) control persons. Since we stopped taking blood after June 2002 for logistic reasons only, this could not have introduced bias. FVIII activity was measured with a mechanical clot detection method on a STA-R coagulation analyzer following the instructions of the manufacturer (Diagnostica Stago, Asnieres, France). Levels of FIX antigen were determined by enzyme-linked immunosorbent assay (ELISA). Von Willebrand factor (VWF) antigen was measured with the immunoturbidimetric method, using the STA Liatest kit (rabbit anti–human VWF antibodies), following the instructions of the manufacturer (Diagnostica Stago).[21,22] The mean intra-assay and inter-assay coefficients of variation were 3.7% and 8.9% for factor VIII activity levels, respectively, 4.3% and 2.7% for factor IX antigen levels, respectively, and 3.6% and 2.6% for von Willebrand factor antigen levels, respectively.

DNA samples were available for 3957 of 4311 (92%) cases and 4680 of 5768 (81%) control persons. Common genetic risk factors were assessed, including the FV Leiden mutation and ABO-blood group, by polymerase chain reactions using the TaqMan assay.[21] To genotype ABO-blood group, we determined the 20146G/- (rs8176719), 21463C/G (rs7853989), 21867A/G (rs8176749), and 21996C/- (rs8176750) blood group polymorphisms by a 5'nuclease assay (Taqman; Applied Biosystems, Foster City, CA, USA) using a standard PCR reaction mix (Eurogentec, Seraing, Belgium) and an allele-specific fluorescent probe equipped with a minorgroove binding moiety (Applied Biosystems). FV Leiden and blood group non-O, and increased levels of VWF, FVIII, and FIX were specifically chosen as thrombophilic conditions, because these are either prevalent genetic risk factors, or coagulation factors associated with highest venous thrombosis risks.[23]

Statistical analysis

To determine whether the presence of one or more major illnesses was associated with an increased risk for venous thrombosis as compared with persons without a major illness, odds ratios with 95% confidence intervals (95% CIs) adjusted for age and sex (the matching factors) were calculated. We made cut-off points for FVIII (155 IU/dL), FIX (119 IU/dL), and VWF levels (142 IU/dL) that correspond with the 80th percentiles in the control population. Persons who were using anticoagulant therapy at time of blood collection were excluded from the analysis

of the effect of vitamin K–dependent coagulation FIX. Odds ratios for venous thrombosis were calculated for the combination of major illness and immobilization, increased body mass index (≥25 kg/m^2), or thrombophilia (FV Leiden, non-O blood group, and elevated levels of FVIII, FIX, and VWF) to identify high-risk groups that could benefit from thromboprophylaxis. Addressing the causal relation between major illness and risk of venous thrombosis was not the aim of this study.[22] Therefore, we only adjusted for the matching factors, i.e. age and sex.[24] Malignancy was not considered as a major illness as a previous report of the MEGA study already published about malignancy and risk of venous thrombosis.[21] Statistical analyses were performed with statistical package SPSS Windows version 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Clinical characteristics

4311 patients with venous thrombosis and 5768 control persons without venous thrombosis were included in the current analysis. Table 1 shows the general characteristics of the study population. Of the patients, 2477 (57%) had a deep vein thrombosis of the leg only, and 1834 (43%) had a pulmonary embolism with or without deep vein thrombosis. Patients, as expected, had a higher BMI, were more often immobilized, were more likely to have FV Leiden and non-O blood group, and had higher levels of coagulation factors than control persons.

Odds ratios for venous thrombosis

Odds ratios for venous thrombosis associated with major illnesses all pointed in the same direction and were of similar magnitude, regardless of whether partners or RDD control persons were used as the control group (Table 2). Therefore, we combined these control groups for the further analyses. The prevalence of a history of a major illness was 14% in patients and 8% in controls. Presence of liver disease (odds ratio 1.7; 95% CI 1.0-2.9), rheumatoid arthritis (odds ratio 1.5; 95% CI 1.2-1.9), heart failure (odds ratio 1.7; 95% CI 1.2-2.3), and arterial thrombosis (odds ratio 1.5; 95% CI 1.2-1.8) were associated with an increased risk of venous thrombosis after adjustment for age and sex. Kidney disease (odds ratio 3.7; 95% CI 2.3-5.9), history of hemorrhagic stroke (odds ratio 4.9; 95% CI 2.4-9.9), and multiple sclerosis (odds ratio 2.4; 95% CI 1.3-4.3) were also associated with an increased risk of venous thrombosis.

Table 1. Baseline characteristics

	Thrombosis patients N= 4311		Partner controls N= 2979		RDD controls N= 2789		Partner and RDD controls N= 5768	
Median age, years (5-95th %)	49.7	(25.9-67.8)	50.4	(28.0-66.4)	45.5	(23.3-67.0)	48.1	(25.4-66.7)
Women, N (%)	2326	(54.0)	1517	(50.9)	1593	(57.1)	3110	(53.9)
Median BMI, kg/m^2, (5-95th %)	26.2	(20.1-35.5)	25.5	(20.3-33.7)	24.5	(19.6-32.5)	25.0	(19.9-33.1)
Immobilization*, N (%)	1662	(38.6)	440	(14.8)	507	(18.2)	947	(16.4)
Thrombophilia								
Factor V Leiden, N (%)	626	(15.8)	143	(5.4)	109	(5.4)	252	(5.4)
Blood group non-O, N (%)	2804	(71.2)	1429	(53.6)	1094	(54.5)	2523	(53.9)
Median factor VIII, IU/dL (5-95th %)	154	(83-280)	116	(65-212)	113	(65-200)	114	(65-208)
Median factor IX, IU/dL (5-95th %)	108	(80-144)	105	(78-139)	102	(76-135)	103	(77-137)
Median VWF, IU/dL (5-95th %)	138	(75-255)	105	(57-196)	103	(57-186)	105	(57-191)

RDD, random digit dialing; BMI, body mass index. *Defined as bedridden for more than 4 days, surgery, or hospitalization within 12 months prior to the index date

Table 2. Association between major illnesses and the risk of venous thrombosis

	Patients	Controls	Adjusted odds ratios* (95% confidence interval)				
	N	N	Overall	Partner controls	RDD controls	Deep vein thrombosis	Pulmonary embolism†
Major illness							
No	3720	5290	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Yes	591	478	1.7 (1.5-1.9)	1.7 (1.5-2.0)	1.6 (1.3-1.9)	1.4 (1.2-1.7)	2.0 (1.7-2.4)
Liver disease	27	22	1.7 (1.0-2.9)	1.8 (0.9-3.7)	1.5 (0.7-3.0)	1.7 (0.9-3.2)	1.6 (0.8-3.3)
Kidney disease	60	23	3.7 (2.3-5.9)	4.1 (2.2-7.9)	3.4 (1.8-6.3)	3.3 (1.9-5.6)	4.2 (2.4-7.2)
Rheumatoid arthritis	145	132	1.5 (1.2-1.9)	1.4 (1.1-1.9)	1.5 (1.1-2.1)	1.3 (1.0-1.8)	1.7 (1.2-2.2)
Multiple sclerosis	30	17	2.4 (1.3-4.3)	4.3 (1.7-11.0)	1.5 (0.8-3.0)	2.6 (1.3-5.0)	2.1 (1.0-4.4)
Heart failure	76	60	1.7 (1.2-2.3)	2.0 (1.3-3.2)	1.3 (0.9-2.0)	1.1 (0.7-1.7)	2.5 (1.7-3.6)
Hemorrhagic stroke	36	10	4.9 (2.4-9.9)	4.6 (1.9-10.9)	5.5 (2.0-15.6)	4.8 (2.2-10.1)	5.0 (2.2-11.2)
Arterial thrombosis	299	264	1.5 (1.2-1.8)	1.6 (1.3-1.9)	1.4 (1.1-1.8)	1.1 (0.9-1.4)	2.0 (1.6-2.4)
MI	137	116	1.5 (1.2-2.0)	1.7 (1.3-2.3)	1.4 (1.0-1.9)	0.9 (0.6-1.3)	2.5 (1.8-3.3)
Angina	64	49	1.7 (1.1-2.4)	1.5 (1.0-2.3)	2.0 (1.2-3.4)	1.1 (0.7-1.8)	2.4 (1.6-3.8)
Ischemic stroke	41	38	1.4 (0.9-2.3)	1.6 (0.9-2.7)	1.3 (0.8-2.3)	1.4 (0.8-2.3)	1.5 (0.9-2.7)
TIA	61	58	1.4 (1.0-2.0)	1.4 (0.9-2.1)	1.4 (0.8-2.2)	1.2 (0.8-1.9)	1.6 (1.0-2.4)
PVD	55	52	1.4 (0.9-2.0)	1.5 (0.9-2.3)	1.3 (0.8-2.1)	1.4 (0.9-2.2)	1.3 (0.8-2.2)

MI, myocardial infarction; TIA, transient ischemic attack; PVD, peripheral vascular disease. *Adjusted for age and sex. †Pulmonary embolism with or without deep vein thrombosis

Odds ratios for deep vein thrombosis and pulmonary embolism

As shown by the point estimates, the odds ratios for deep vein thrombosis alone were higher than the odds ratios for pulmonary embolism with or without deep vein thrombosis in patients with liver disease and multiple sclerosis. All other major illnesses yielded a similar or lower odds ratio for deep vein thrombosis alone than for pulmonary embolism with or without deep vein thrombosis.

Combined major illnesses

The odds ratio of venous thrombosis was 2.2 (95% CI 1.5-3.1) in the presence of two or more major illnesses and was 1.6 (95% CI 1.4-1.8) in the presence of only one major illness as compared to the absence of a major illness.

Immobilization, body mass index and thrombophilia

In Table 3, the combined effects of immobilization, increased body mass index (≥25 kg/m^2) or thrombophilia with major illnesses on venous thrombotic risk are shown. The odds ratio for immobilization in the absence of major illnesses was 6.2 (95% CI 5.4-7.0). The combination of immobilization with a major illness yielded an odds ratio of 10.4 (95% CI 7.5-14.4). Participants with liver disease, kidney disease, rheumatoid arthritis, and arterial thrombosis had odds ratios in combination with immobilization of 8.3 (95% CI 2.8-24.4), 31.7 (95% CI 7.6-132.1), 11.7 (95% CI 5.8-23.6) and 11.6 (95% CI 7.1-19.2), respectively. The odds ratio of venous thrombosis for a major illness was 2.3 (95% CI 1.9-2.9) in the absence of an increased body mass index and 2.3 (95% CI 2.0-2.8) in the presence of an increased body mass index. The odds ratio of venous thrombosis for a major illness was 1.7 (95% CI 1.5-2.0) without FV Leiden and 4.0 (95% CI 2.5-6.5) in FV Leiden carriers. The odds ratio of venous thrombosis for a major illness in the presence of blood group non-O was 3.3 (95% CI 2.7-4.0) and the odds ratio in the absence of blood group non-O was 1.9 (95% CI 1.5-2.4). A major illness combined with a normal level of FVIII, FIX, or VWF led to odds ratios of 1.6 (95% 1.2-2.1), 1.4 (95% 1.1-1.8), and 1.6 (95% 1.2-2.0), respectively. These odds ratios were 5.5 (95% CI 4.1-7.3), 2.3 (95% CI 1.7-3.2), and 5.4 (95% CI 4.1-7.3) in the presence of elevated levels of FVIII, FIX, or VWF, respectively. Participants with multiple sclerosis in combination with increased FVIII levels had a high risk of venous thrombosis (odds ratio 12.5; 95% CI 1.5-107.9), as did participants with arterial thrombosis and increased FVIII levels (odds ratio 5.5; 95% CI 3.8-8.0).

Table 3. Combined effect of established risk factors for venous thrombosis and major illnesses on venous thrombosis risk

Major illness	Risk factor	Immobilization		BMI ≥25 kg/m^2		Factor V Leiden		Blood group non-O		FVIII		FIX		VWF	
		OR*	(95% CI)	OR*	(95% CI)	OR*	(95% CI)	OR*	(95% CI)	OR*	(95% CI)	OR*	(95% CI)	OR*	(95% CI)
No major illness	No risk factor	1	(ref)	1	(ref)	1	(ref)	1	(ref)	1	(ref)	1	(ref)	1	(ref)
Major illness	No risk factor	1.3	(1.2-1.6)	2.3	(1.9-2.9)	1.7	(1.5-2.0)	1.9	(1.5-2.4)	1.6	(1.2-2.1)	1.4	(1.1-1.8)	1.6	(1.2-2.0)
No major illness	Risk factor	6.2	(5.4-7.0)	1.7	(1.5-1.8)	3.5	(3.0-4.1)	2.2	(2.0-2.4)	4.5	(3.9-5.2)	1.6	(1.4-1.9)	4.2	(3.7-4.8)
Major illness	Risk factor	10.4	(7.5-14.4)	2.3	(2.0-2.8)	4.0	(2.5-6.5)	3.3	(2.7-4.0)	5.5	(4.1-7.3)	2.3	(1.7-3.2)	5.4	(4.1-7.3)
Liver disease	No risk factor	0.9	(0.4-2.0)	2.8	(1.1-6.8)	1.6	(0.8-3.1)	4.1	(1.0-16.3)	1.5	(0.3-6.9)	1.3	(0.5-3.4)	0.6	(0.1-2.9)
	Risk factor	8.3	(2.8-24.4)	2.0	(0.9-4.6)	5.9	(0.7-52.6)	2.2	(1.0-4.5)	4.5	(1.8-11.6)	11.0	(1.3-91.5)	8.0	(2.6-24.7)
Kidney disease	No risk factor	2.1	(1.2-3.9)	5.0	(2.3-10.8)	3.6	(2.1-6.1)	3.8	(1.8-7.9)	2.8	(0.9-8.4)	1.8	(0.8-4.4)	1.8	(0.8-4.5)
	Risk factor	31.7	(7.6-132.1)	4.9	(2.6-9.1)	4.3	(0.9-21.4)	7.0	(3.4-14.1)	3.9	(1.7-9.0)	1.8	(0.5-6.2)	7.2	(2.3-22.3)
Rheumatoid arthritis	No risk factor	1.2	(0.9-1.6)	2.1	(1.5-3.1)	1.5	(1.2-2.0)	2.1	(1.4-3.1)	1.2	(0.7-2.1)	1.2	(0.8-2.0)	1.4	(0.8-2.2)
	Risk factor	11.7	(5.8-23.6)	1.8	(1.3-2.6)	3.0	(1.3-7.5)	2.6	(1.8-3.7)	5.1	(3.0-8.7)	2.2	(1.2-4.0)	4.8	(2.8-8.3)
Multiple sclerosis	No risk factor	2.5	(1.2-5.5)	2.0	(0.8-4.9)	2.6	(1.4-5.1)	2.7	(1.0-6.8)	2.1	(0.7-5.7)	1.7	(0.6-5.0)	2.3	(0.8-6.6)
	Risk factor	4.3	(1.6-11.2)	5.1	(2.2-12.2)	1.3	(0.1-20.4)	4.8	(2.0-11.6)	12.5	(1.5-107.9)	4.6	(0.9-23.6)	5.8	(1.1-29.9)
Heart failure	No risk factor	1.7	(1.1-2.5)	2.9	(1.6-5.2)	1.8	(1.2-2.6)	1.6	(0.9-2.9)	1.6	(0.8-3.2)	1.1	(0.5-2.3)	1.7	(0.9-3.3)
	Risk factor	4.6	(2.3-9.2)	2.0	(1.3-3.1)	2.0	(0.3-12.2)	3.5	(2.2-5.7)	6.9	(3.2-15.1)	2.3	(0.9-5.8)	6.7	(3.0-15.3)
Hemorrhagic stroke	No risk factor	3.3	(1.5-7.2)	12.8	(2.9-56.3)	4.6	(2.2-9.8)	1.4	(0.5-4.5)	7.6	(2.1-28.4)	4.1	(1.3-13.5)	5.9	(1.8-19.0)
	Risk factor	Not estimable		5.9	(2.5-13.9)	Not estimable		26.5	(6.3-112.1)	6.7	(1.8-25.5)	6.9	(0.8-61.6)	8.6	(1.8-41.9)
Arterial thrombosis	No risk factor	1.2	(1.0-1.5)	2.0	(1.5-2.8)	1.5	(1.2-2.8)	1.7	(1.3-2.3)	1.6	(1.2-2.3)	1.6	(1.1-2.3)	1.6	(1.2-2.3)
	Risk factor	11.6	(7.1-19.2)	2.2	(1.7-2.7)	5.2	(2.6-10.5)	3.0	(2.3-3.8)	5.5	(3.8-8.0)	2.2	(1.4-3.3)	5.0	(3.4-7.2)

OR, odds ratio; VWF, von Willebrand factor. *Odds ratio adjusted for age and sex. Thrombophilia defined as levels elevated above the 80th percentiles of the control group: FVIII levels >155 IU/dL, FIX >119 IU/dL, and VWF >142 IU/dL

Combinations of a major illness with immobilization and increased FVIII levels (odds ratio 79.9; 95% CI 33.2-192.2), increased FIX levels (odds ratio 35.3; 95% CI 14.2-87.8), increased VWF levels (odds ratio 88.0; 95% CI 33.9-228.3), FV Leiden (odds ratio 84.2; 95% CI 19.5-363.6), and blood group non-O (odds ratio 53.1; 95% CI 30.9-91.4) were associated with the highest venous thrombosis risks (Table 4).

Table 4. Odds ratios for venous thrombosis for combinations of risk factors

Major illness	Immobilization	Thrombophilia*	Age and sex adjusted odds ratio (95% CI)
No major illness	No immobilization	No Thrombophilia	1 (ref)
Any major illness	+ immobilization	No Thrombophilia	10.9 (4.2-28.2)
Any major illness	+ immobilization	+ increased FVIII levels	79.9 (33.2-192.2)
Any major illness	+ immobilization	+ increased FIX levels	35.3 (14.2-87.8)
Any major illness	+ immobilization	+ increased VWF levels	88.0 (33.9-228.3)
Any major illness	+ immobilization	+ factor V Leiden	84.2 (19.5-363.6)
Any major illness	+ immobilization	+ blood group non-O	53.1 (30.9-91.4)

*No thrombophilia defined as the absence of increased FVIII levels (>155 IU/dL), increased FIX levels (>119 IU/dL), increased VWF levels (>142 IU/dL), factor V Leiden, and blood group non-O.

DISCUSSION

In this large case-control study, major illnesses were associated with an overall 1.7-fold (95% CI 1.5-1.9) increased risk of venous thrombosis, varying from a 1.4-fold increased risk for a history of ischemic stroke, transient ischemic attacks and peripheral vascular disease to a 4.9-fold increased risk for hemorrhagic stroke. Overall, odds ratios were slightly higher for pulmonary embolism with or without deep vein thrombosis than for deep vein thrombosis alone. Major illnesses were associated with higher risks of venous thrombosis when the patient was additionally immobilized and had thrombophilia. This was not the case for the combination of a major illness with an increased body mass index.

The reason for performing this study was to explore whether risk groups could be identified that would benefit from targeted prevention of venous thrombosis with pharmacological agents. Therefore, we were not interested in the causal relation between major illnesses and venous thrombosis, i.e. we did not adjust for potential confounding factors. From a prediction point of view, it is not important whether a major illness has a causal relation with venous thrombosis, since these patients could be targeted for thromboprophylaxis if there is a high risk of venous thrombosis irrespective of whether a causal relation exists or not.[22]

On the whole, based on the point estimates, our study suggests that the risk of venous thrombosis was increased when more major illnesses were present or when a major illness was present in combination with immobilization and thrombophilia. This is in line with the concept of venous thrombosis as a multicausal disease.[25] The presence of more major illnesses could have increased coagulation factor levels in MEGA participants. In addition, immobilization may be a good marker for severity of disease. As we had no information on disease severity, we could not investigate whether the underlying major illness itself, or immobilization associated with major illness, increased the risk of venous thrombosis. However, for prediction of high-risk groups, the causal path leading to venous thrombosis is not important as, either way, preventive measures can be initiated when the risk is deemed to outweigh the side-effects. Overall, our study suggests that many major illnesses in association with immobilization increase the risk of venous thrombosis.

That the risk for venous thrombosis was higher in participants with a history of hemorrhagic stroke (odds ratio 4.9; 95% CI 2.4-9.9) than in participants with a history of ischemic stroke (odds ratio 1.4; 95% CI 0.9-2.3) deserves additional comment. This finding might represent the less frequent use of thromboprophylaxis in patients with hemorrhagic stroke. In most epidemiological studies, hemorrhagic and ischemic stroke are combined.[4,18] However, one other study also found an higher risk of venous thrombosis in patients with hemorrhagic stroke than in patients with ischemic stroke.[14]

We defined immobilization as being bedridden at home for at least 4 days, being hospitalized, or having surgery within three months prior to the index date. We found high odds ratios for venous thrombosis for immobilization in combination with liver disease (odds ratio 8.3; 95% CI 2.8-24.4), kidney disease (odds ratio 31.7; 95% CI 7.6-132.1), rheumatoid arthritis (odds ratio 11.7; 95% CI 5.8-23.6) and arterial thrombosis (odds ratio 11.6; 95% CI 7.1-19.2). ACCP guidelines currently do not consider thromboprophylaxis for immobilized patients with these conditions. If other studies confirm our new findings, intervention trials for weighing the benefits and risks of thromboprophylaxis are recommended for these high-risk groups with a major illness.

It is important to note that the baseline risk of venous thrombosis in absence of immobilization is low (less than 1.4 per 1000 persons per year).[7] Therefore odds ratios of 2.1 (for kidney disease in the absence of immobilization), 2.5 (for multiple sclerosis in the absence of immobilization), 1.7 (for heart failure in the absence of immobilization), 3.3 (for hemorrhagic stroke in the absence of immobilization), and 1.2 (for arterial thrombosis in the absence of immobilization) would probably result in an absolute risk of venous thrombosis of less than 4.6 (3.3 times 1.4) per 1000 persons per year in patients with these major illnesses. Thus, if

patients with any of these major illnesses were treated with long term thromboprophylaxis, the number needed to treat would be excessively high, while introducing a considerable risk of major bleeding. For this reason, thromboprophylaxis in patients with a major illness seems unjustified when no additional risk situations, such as hospitalization or surgery, are present.

An intriguing observation in our study was that myocardial infarction and angina were more clearly associated with pulmonary embolism than with deep vein thrombosis. Other studies also showed that the risk of pulmonary embolism was higher than for deep vein thrombosis in persons with such comorbidity.[4,18] This observation may be due to misclassification, which is possible as signs and symptoms of arterial thrombosis can be similar to pulmonary embolism. Alternatively, it may also be a causal observation as angina or myocardial infarction reflect local inflammatory effects in the lungs, which may lead to an increased risk of pulmonary embolism.[26]

The strengths of this study include the large patient sample, the detailed information about immobilization in both patients and controls, and the combination with data on thrombophilia. A limitation of this study is that major illnesses were assessed via self-report and the exact diagnoses of these major illnesses were not available. In addition, no specific questions were asked about the (severity of) major illnesses. However, since these are major diseases with a large impact, we expect that both patients and control persons reported their illnesses to a similar extent, thus limiting recall bias. Any resulting random misclassification would lead to an underestimation of our odds ratios. A second limitation of this study is that the blood sample was collected after the thrombotic event. Therefore, we cannot exclude the possibility that differences in plasma levels of the coagulation factors between cases and control persons were the result of the thrombotic event itself. However, the blood draw was performed at least 3 months after the thrombotic event, diminishing the possibility that the thrombotic event itself caused abnormalities in coagulation factor levels through acute phase reactions. A third limitation was that, as we had no data on the time between major illness and the subsequent venous thrombotic event in our study, we could not calculate specific risk estimates for different time frames. Not many previous studies were able to calculate a risk of venous thrombosis after major illness diagnosis, specified on time. However, the one that could showed that the risk was highest shortly after the major illness was diagnosed.[18] Therefore, our relative risk estimates would probably have been higher if we could have taken this time aspect into close consideration. A fourth limitation was that for participation as a case in MEGA, those who died soon after a first venous thrombotic event (4% of the patient population) were excluded.[20] This probably has led to an underestimation of our risk estimates, since patients with a major illness are more likely to die from venous thrombosis than patients without a major illness. A fifth limitation of our study was that we had limited power for several analyses. This low power of the study may have led to some unexpected findings, like a higher risk of venous

thrombosis among participants with angina than among participants with prior ischemic stroke. This finding should therefore be interpreted with caution. A sixth limitation is that we had no information about thromboprophylaxis during immobilization. This probably led to an underestimation of our risk estimates, as it is likely that patients with a major illness receive more thromboprophylaxis than other persons. Another aspect was that the risk of venous thrombosis could be especially increased for particular conditions causing liver disease, kidney disease, or heart failure. However, we did not have this information in our study. Future studies are needed on this topic. A final aspect of our study was that we had two separate control groups. However, by having a control group partly consisting of partners of patients, we probably have conservative estimates as partners will be more likely to resemble the cases than random controls. Therefore, we would expect higher odds ratios when comparing to RDD controls than to partners. Nevertheless, results pointed in the same direction and were roughly similar when both control groups were analyzed separately. Therefore, we do not think that our results are affected by the two different control groups.
In summary, we have reported a detailed epidemiological analysis on the risk of first venous thrombosis in patients with a major illness. All major illnesses reported here were associated with an increased risk of venous thrombosis ranging from an odds ratio of 1.5 for rheumatoid arthritis and arterial thrombosis to 4.9 for hemorrhagic stroke. These risks were most pronounced at time of immobilization and in the presence of thrombophilia. These results could be a guide for future thromboprophylaxis decisions.

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Chapter 3

Chronic kidney disease stage 1-3 increases risk of venous thrombosis

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ABSTRACT

Background: End-stage renal disease has been associated with venous thrombosis (VT). However, the risk of VT in early stages of chronic kidney disease (CKD) has not yet been investigated. The aim of this study was to investigate whether CKD patients with stage 1-3 are at increased risk of VT.

Methods: 8 495 subjects were included in a prospective cohort study, in which renal function and albuminuria was assessed, starting in 1997-1998, and were followed for the occurrence of VT until 1 June 2007. CKD patients were staged according to the Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines, on the basis of 24-h urine albumin excretion and estimated glomerular filtration rates. Objectively verified symptomatic VT was considered as endpoint.

Results: Of the 8 495 subjects, 243 had CKD stage 1, 856 CKD stage 2, and 491 CKD stage 3. During a median follow-up period of 9.2 years, 128 individuals developed VT. The hazard ratios (HRs) for CKD stages 1, 2, and 3 were, respectively, 2.2 (95% CI 0.9-5.1), 1.9 (95% CI 1.1-3.1), and 1.6 (95% CI 0.9-2.8) relative to those without CKD after adjustment for age, sex, BMI, hypertension, diabetes, malignancy, and high-sensitivity C-reactive protein. Subjects with CKD stage 3 and albuminuria (≥30 mg per day) had an adjusted HR of 3.0 and subjects with stage 3 without albuminuria had an adjusted HR of 1.0.

Conclusion: CKD stages 1 and 2, and CKD stage 3 in presence of albuminuria are risk factors for VT. The risk of VT is more related to albuminuria than to impaired glomerular filtration rate.

INTRODUCTION

Patients with severe chronic kidney disease (CKD) have increased risks of both arterial cardiovascular disease as well as for venous thrombosis (VT). The Kidney Disease Outcome Quality Initiative (K/DOQI) guidelines defined CKD as either kidney damage (albuminuria ≥30 mg per day) or decreased kidney function and categorized CKD in five stages.[1,2] The prevalence of CKD in the US is now 13% and is increasing, predominantly as a result of the type II diabetes epidemic.[3]

The increased risk of arterial cardiovascular disease in CKD has been known for a long time and has been studied extensively for different CKD stages.[4-8] Recent studies have also shown an association between overt CKD and VT.[9,10] A study of the PREVEND cohort showed that the presence of micro-albuminuria (albuminuria 30-300 mg per day) was a risk factor for VT.[9] Another study of the LITE cohort showed that patients with estimated glomerular filtration rates (eGFR) between 15 and 60 ml/min (CKD stage 3-4) had a two-fold increased risk of VT as compared to subjects with a normal kidney function (eGFR >90 ml/min).[10] However, information on albuminuria was not available in this study. To our knowledge, there is no study on the risk of VT in the different CKD stages taking into account albuminuria which is a prerequisite for staging CKD and for defining patients without CKD.

Therefore, we investigated whether patients with CKD stage 1, 2, and 3 had an increased risk of VT in a large population-based cohort, and set out to determine absolute and relative risks for various stages of CKD.

METHODS

Study population and design

For this study, we used data of PREVEND study, which was designed to investigate the association between albuminuria and renal and cardiovascular outcomes in the general population. Details of the study have been published elsewhere[11-13] and can be found at http://www.prevend.org. The study outline is presented in Figure 1. In summary, all inhabitants of the city of Groningen, the Netherlands, aged 28-75 years (n= 85 421) were invited to send a morning urine sample to screen for albuminuria. Of these subjects, 40 856 responded. From these responders, the PREVEND cohort was selected aiming for a cohort enriched for the presence of albuminuria. Pregnant women and subjects with insulin-dependent diabetes mellitus were excluded. All participants with an urinary albumin concentration (UAC) of ≥10 mg/L were invited (N=9 966), of whom 6 000 subjects participated. Furthermore, a randomly selected cohort group of

2 592 subjects selected from 30 890 respondents with UAC of <10 mg/L participated. These 8 592 subjects formed the baseline PREVEND cohort. These participants twice visited an outpatient clinic for measurements concerning their health. For the current study, subjects were excluded because of missing data on 24-h urinary albumin excretion or creatinine (n=86). Furthermore, subjects with CKD stage 4 (n=8) or stage 5 (n=3) were excluded, one of whom had a VT event, leaving 8 495 subjects for the present analysis. The PREVEND study has been approved by the local medical ethics committee and is conducted in accordance with the guidelines of the Declaration of Helsinki.

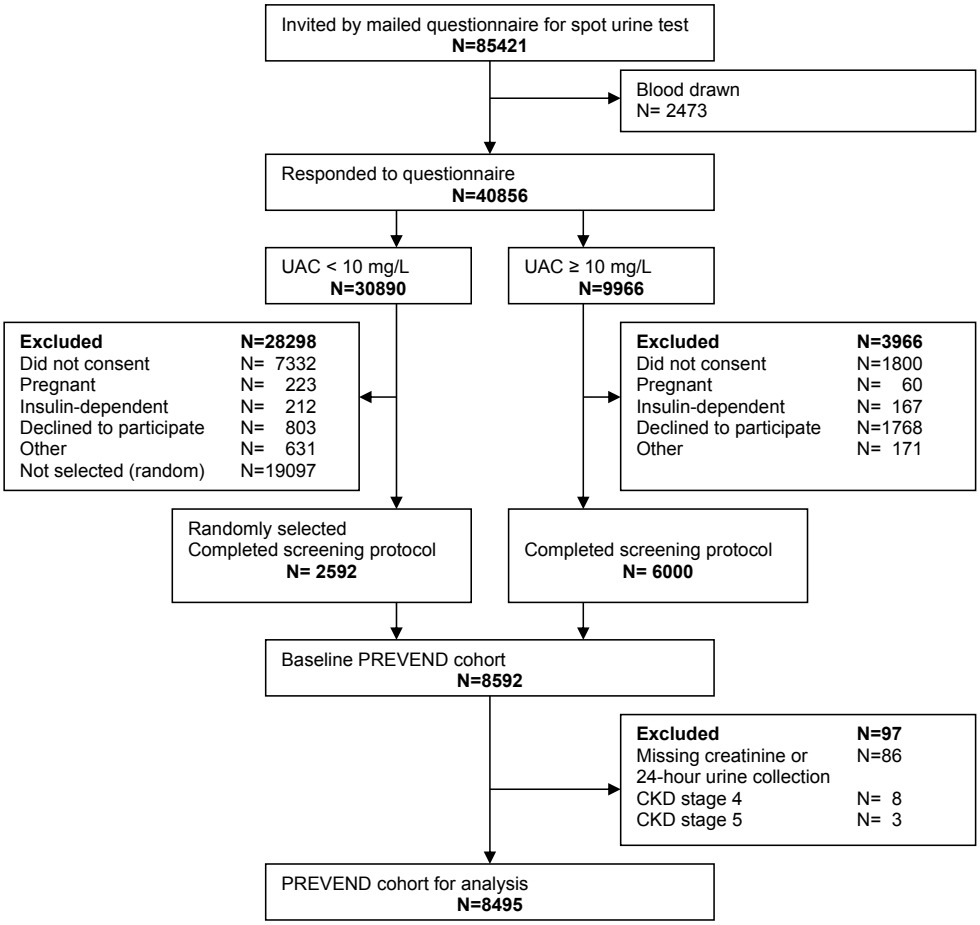

Figure 1. Outline of the PREVEND study
CKD, chronic kidney disease; UAC, urinary albumin concentration

Measurements and definitions

Serum creatinine, total cholesterol, and plasma glucose were measured by dry chemistry (Eastman Kodak, Rochester, New York). The high-sensitivity C-reactive protein (hsCRP) level was determined by nephelometry (BN II, Dade Behring, Marburg, Germany). Participants collected two 24-h urine samples, in which UAC was determined by nephelometry (BN II, Dade Behring, Marburg, Germany). The amount of albuminuria was measured as the mean of the two 24-hour urine samples.

Hypertension was defined as systolic blood pressure of ≥140 mm Hg, diastolic blood pressure of ≥90 mm Hg, or the use of antihypertensive drugs. Diabetes was defined as a fasting glucose level of ≥126 mg/dL, a non-fasting plasma glucose levels of ≥200 mg/dL, or the use of oral antidiabetic drugs. Hypercholesterolemia was defined as a total serum cholesterol concentration ≥250 mg/dL, or in case of a previous myocardial infarction or stroke a concentration of ≥193 mg/dL, or the use of lipid-lowering drugs. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. GFR was estimated by the Modification of Diet in Renal Disease (MDRD) study equation[14] taking into account sex, age, race, and serum creatinine level. In an additional analysis, the newly developed but less often used Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) study equation[15] was used to estimate eGFR to compare these results with the results of the MDRD-equation. The CKD-EPI equation has been shown to outperform the MDRD equation in estimating the GFR above the 60 ml/min.[15]

Chronic kidney disease

CKD was staged according to the K/DOQI guidelines.[1,2] CKD stage 1 was defined as eGFR >90 ml/min and albuminuria (urinary albumin excretion ≥30 mg per 24-hour urine collection), CKD stage 2 as eGFR between 60 and 90 ml/min and albuminuria, and CKD stage 3 as eGFR between 30 and 60 ml/min.

Venous thrombosis

The regional anticoagulation clinic database was used to identify participants who developed VT between January 1997 and June 2007. In the Netherlands, all outpatient treatment with vitamin K antagonists is monitored by regional anticoagulation clinics. Therefore, all VT events in treated outpatients are recorded by anticoagulation clinics. Moreover, as a secondary check for outpatient VT cases and identification of within hospital (fatal) cases, all study participants were searched for VT events in the national registry of death certificates and the national registry of hospital discharge diagnoses datasets. With the use of three independent sources, it is unlikely that VT events will be missed. The investigators who collected these data were blinded for CKD stages of the participants. In addition, all VT events according

to the three sources were validated by reviewing medical records of these patients. Only objectively verified symptomatic VT events were considered. Deep vein thrombosis (DVT) was confirmed by compression ultrasound and pulmonary embolism (PE) by ventilation-perfusion lung scanning, spiral computed tomography, or at autopsy. The observation time of each participant was calculated as a time elapsed between the testing of albuminuria (1997-1998) and the first episode of VT or a censoring event (withdrawal from the study, moving out of the city, death, or June 2007), whichever occurred first. Incidence rates for VT were calculated by dividing the number of patients with a VT by the total observation time at risk. VT was considered to be unprovoked in the absence of major surgery, trauma, immobilization for >7 days, oral contraceptives, hormone therapy, pregnancy, malignant disease, long-distance travel for >4 hours, active infectious disease, paresis/paralysis of the leg, or heart failure at or within three months before the development of VT. Medical records were viewed with a checklist including these well-defined and well-documented variables to categorize VT into provoked or unprovoked.

Statistical analyses

Baseline characteristics of the participants were compared between subjects without CKD and subjects with CKD stages 1-3. Continuous data were reported as medians with interquartile ranges. Kaplan-Meier life-tables were used to estimate cumulative survival for CKD stages 1-3 and no CKD. To investigate whether patients with CKD stages 1-3 had an increased risk of VT, proportional hazard regression was used to calculate hazard ratios (HRs) with 95% confidence intervals (CIs) as compared to participants without CKD (reference group). All analyses were performed for CKD stages 1, 2, and 3 combined and separately. In contrast to CKD stages 1 and 2, CKD stage 3 is only defined by decreased eGFR (between 30 and 60 ml/min) and not by the presence of albuminuria, according to the K/DOQI guidelines. We also calculated HRs for CKD stage 3 stratified for the presence of albuminuria. We adjusted the HRs for age, sex, and BMI and additional for hypertension, diabetes, malignancy, and hsCRP. HRs were not adjusted for other cardiovascular risk factors such as hyperlipidemia and smoking, since these were not associated with VT in the PREVEND cohort.[9] We repeated the same analyses for provoked and unprovoked VT separately.

To investigate whether eGFR is a risk factor for VT apart from albuminuria, we calculated HRs with 95% CIs for eGFR adjusted for albuminuria and for albuminuria adjusted for eGFR to evaluate the associations of level of eGFR and albuminuria with risk of VT. Furthermore, we divided subjects in six categories based on albuminuria and eGFR (>90 ml/min, between 60 and 90 ml/min, and between 30 and 60 ml/min). HRs with 95% CIs were calculated for eGFR in absence or presence of albuminuria as compared with subjects with eGFR >90 ml/min without albuminuria (reference group).

Finally, we calculated HRs with 95% CIs for CKD stages 1-3 as compared to participants without CKD, using the CKD-EPI formula for staging CKD. STATA software version 10.1 (StataCorp LP, College Station, Tx) was used for the statistical analyses.

RESULTS

The baseline characteristics of the 8 495 subjects are shown in Table 1. Of the 6 905 subjects without CKD, 26.4% had an eGFR >90 ml/min and 73.6% had an eGFR between 60 and 90 ml/min. Of the 1 590 with CKD, 243 were in stage 1, 856 in stage 2, and 491 in stage 3. Of the 491 subjects with stage 3 CKD, 164 had albuminuria (≥30 mg per day). Subjects with CKD stages 1-3 were older, were more often male, more often had diabetes, hypertension and malignancy, and had a higher BMI and higher CRP levels than subjects without CKD. The age of CKD patients increased with the CKD stage.

Overall, 128 subjects developed VT during a median observation period of 9.2 years (ranging from 0 to 10 years). Of the 128 patients with VT, 72 (56%) had DVT only, 44 had PE only (34%), and 12 (9%) had a combination of both. Of the 1590 subjects with CKD stage 1-3, 49 developed VT as compared with 79 of the 6905 subjects without CKD. Seven of the 243 patients with CKD stage 1, 26 of the 856 patients with CKD stage 2, and sixteen of 491 patients with CKD stage 3 developed VT. Four patients died because of a PE (three in CKD stage 3 and one without CKD). Furthermore, there was no significant difference in the distribution of PE and DVT in CKD stage 3 (63% of VT patients had a PE) as compared to CKD stages 1 and 2 (36% had a PE) (P=0.09) or as compared to no CKD (43% had a PE) (P=0.16). The cumulative incidences for VT at eight years of follow-up were 3.2% for CKD stage 1, 3.0% for stage CKD 2, 3.3% for CKD stage 3, 3.1% for CKD stages 1-3, and 1.1% for no CKD. The number needed to treat to prevent one VT event in patients with CKD stage 1-3 was approximately 400 patients per year. Figure 2 shows the Kaplan-Meier risk curves for VT events for patients with CKD stage 1-3 versus subjects without CKD.

Table 1. Baseline characteristics

Characteristic	No CKD (n=6905)	CKD stage 1-3 (n=1590)	CKD stage 1 (n=243)	CKD stage 2 (n=856)	CKD stage 3 (n=491)
Age* (years)	46 (37-56)	59 (48-67)	47 (39-56)	58 (48-66)	65 (58-70)
Male, %	49	56	66	64	38
Caucasians, %	95	96	93	97	97
Diabetes, %	2.4	9.8	12.8	11.2	5.9
Hypertension, %	27	65	50	65	72
Hypercholesterolemia, %	28	46	36	44	54
BMI* (kg/m^2)	25 (23-28)	27 (24-30)	27 (24-30)	27 (25-30)	27 (25-30)
hsCRP* (mg/L)	1.1 (0.5-2.7)	2.2 (1.0-4.6)	2.1 (0.9-4.9)	2.3 (1.0-4.4)	2.2 (1.1-4.8)
Malignancy, %	1.4	2.3	1.6	2.2	2.8
eGFR* (ml/min)	81 (73-91)	72 (59-83)	97 (93-104)	76 (69-82)	55 (51-58)
UAE* (mg per day)	8 (6-12)	47 (33-93)	57 (39-101)	59 (39-107)	14 (7-47)

CKD, chronic kidney disease; BMI, body mass index; hsCRP, high-sensitivity c-reactive protein; eGFR, estimated glomerular filtration rate; UAE, urinary albumin excretion. *median (interquartile range)

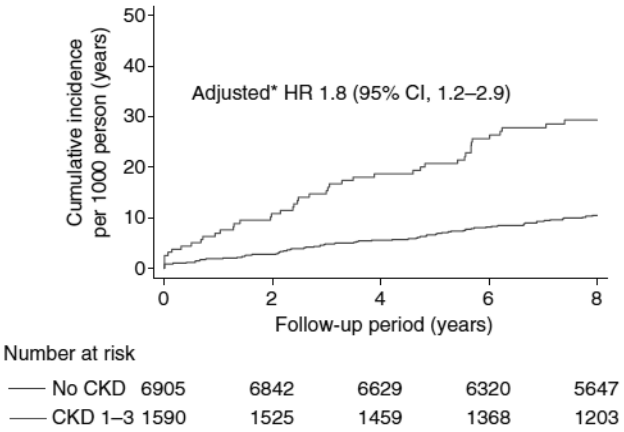

Figure 2. **Kaplan-Meier estimates of the risk of venous thrombosis according to stages of chronic kidney disease**
CKD, chronic kidney disease; prs-yrs, person-years; yrs, years. *Adjusted for age, sex, body mass index, hypertension, diabetes, malignancy, and hsCRP

The incidence rate for VT in subjects without CKD was 1.3 (95% CI 1.1-1.7) per 1000 person-years and 3.7 (95% CI 2.8-4.0) for subjects with CKD stages 1-3 with a corresponding HR for VT of 2.8 (95% CI 2.0-7.3) for CKD stages 1-3 as compared with no CKD. The HR decreased to 1.8 (95% CI 1.2 -2.9) after adjustment for age, sex, BMI, hypertension, diabetes, malignancy, and hsCRP.

The crude HRs were 2.6 (95% CI 1.2-5.6), 2.8 (95% CI 1.8-4.3), and 3.0 (95% CI 1.8-5.2) for respectively CKD stages 1, 2, and 3. Figure 3 shows adjusted HRs with 95% CIs for CKD stages 1, 2, and 3, the last with or without the presence of albuminuria as compared with no CKD. The HRs were 2.2 (95% CI 0.9 -5.1), 1.9 (95% CI 1.1 -3.1), and 1.6 (95% CI 0.9 -2.8). For CKD stage 3 with and without albuminuria, the HRs were, respectively, 5.5 (95% CI 2.8-11.0) and 1.9 (95% CI 0.9-4.2) without adjustment, and 3.0 (95% CI 1.4-6.5) and 1.0 (95% CI 0.4-2.4) after full adjustment.

Of the 128 VT events, 66 were unprovoked (51.6%) and 62 (48.4%) were provoked (Table 2). For unprovoked VT, the HRs after adjustment for age, sex, BMI, hypertension, diabetes, malignancy, and hsCRP were 2.1 (95% CI 1.2-3.6) for CKD stages 1-3, 2.5 (95% CI 0.8-7.4) for CKD stage 1, 2.4 (95% CI 1.3-4.4) for CKD stage 2, and 1.4 (95% CI 0.6-3.3) for CKD stage 3. For provoked VT, the HRs after adjustment were 1.2 (95% CI 0.6-2.3) for CKD stages 1-3, 1.4 (95% CI 0.3-5.9) for CKD stage 1, 0.8 (95% CI 0.3-2.2) for CKD stage 2, and 1.7 (95% CI 0.8-3.9) for CKD stage 3.

Albuminuria was associated with a 2.1-fold increased risk of VT after adjustment for age, sex, BMI, hypertension, diabetes, malignancy, hsCRP, and eGFR (Table 3). As compared with subjects with an eGFR >90 ml/min, subjects with an eGFR between 30 and 60 ml/min had 50% increased risk of VT after adjustment for after adjustment for age, sex, BMI, hypertension, diabetes, malignancy, hsCRP, and albuminuria.

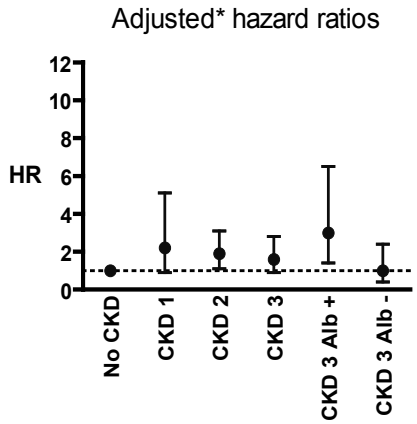

Figure 3. Adjusted hazard ratios for venous thrombosis by CKD stage
CKD, chronic kidney disease; HR, hazard ratio; eGFR, estimated glomerular filtration rate; CKD 3 Alb +, CKD stage 3 and urinary albumin excretion ≥30 mg per day; CKD 3 Alb -, CKD stage 3 and urinary albumin excretion <30 mg per day. *Adjusted for age, sex, body mass index, hypertension, diabetes, malignancy, and hsCRP

Table 2. Incidence rates and hazard ratios for provoked and unprovoked venous thrombosis

	No CKD (n=6905)	CKD stage 1-3 (n=1590)	CKD stage 1 (n=243)	CKD stage 2 (n=856)	CKD stage 3 (n=491)
Unprovoked venous thrombosis					
No. of venous thrombosis	35	31	5	19	7
Incidence rate per 1000 person-years	0.6 (0.4-0.8)	2.4 (1.7-3.4)	2.5 (1.0-5.9)	2.7 (1.7-4.2)	1.7 (0.8-3.7)
Crude hazard ratios (95% CI)	1.0	4.0 (2.5-6.5)	4.2 (1.6-10.6)	4.5 (2.6-7.9)	3.0 (1.3-6.7)
Adjusted* hazard ratios (95% CI)	1.0	2.1 (1.5-3.5)	3.0 (1.1-.8.0)	2.3 (1.3-4.2)	1.4 (0.6-3.2)
Adjusted† hazard ratios (95% CI)	1.0	2.1 (1.2-3.6)	2.5 (0.8-.7.4)	2.4 (1.3-4.4)	1.4 (0.6-3.3)
Provoked venous thrombosis					
No. of venous thrombosis	44	18	2	7	9
Incidence rate per 1000 person-years	0.7 (0.5-1.0)	1.4 (0.9-2.2)	1.0 (0.2-3.9)	1.0 (0.5-2.1)	2.3 (1.2-4.4)
Crude hazard ratios (95% CI)	1.0	1.9 (1.1-3.2)	1.3 (0.3-5.5)	1.3 (0.6-3.0)	3.1 (1.5-6.3)
Adjusted* hazard ratios (95% CI)	1.0	1.2 (0.7-2.2)	1.2 (0.3-4.9)	1.0 (0.4-2.2)	1.7 (0.8-3.6)
Adjusted† hazard ratios (95% CI)	1.0	1.2 (0.6-2.3)	1.4 (0.3-5.9)	0.8 (0.3-2.2)	1.7 (0.8-3.9)

CKD, chronic kidney disease. *Adjusted for age, sex, and body mass index. †Adjusted for age, sex, body mass index, hypertension, diabetes, malignancy, and hsCRP

Table 4 shows HRs for VT for decreased eGFR (between 60 and 90 ml/min and between 30 and 60 ml/min) in absence and presence of albuminuria as compared with subjects with eGFR >90ml/min without albuminuria. The adjusted HRs for subjects without albuminuria and an eGFR between 60 and 90 ml/min or an eGFR between 30 and 60 ml/min were, respectively, 1.5 (95% CI 0.7-3.1) and 1.4 (95% CI 0.5-4.1). HRs for VT were increased in the presence of albuminuria in all eGFR categories. The adjusted HRs were 3.1 (95% CI 1.1-8.9), 2.7 (95% CI 1.2-6.1), and 4.1 (95% CI 1.5-11.0) for subjects with albuminuria and, respectively, eGFR >90 ml/min, eGFR between 60 and 90 ml/min, and eGFR between 30 and 60 ml/min.

The HRs for VT in CKD stages 1, 2, and 3 were, respectively, 1.6 (95% CI, 0.7-3.8), 1.9 (95% CI, 1.2-3.0), and 1.5 (95% CI, 0.9-2.7) using the CKD-EPI formula after adjustment for age, sex, and BMI. HRs for subjects with CKD stage 3 and albuminuria and subjects with CKD stage 3 without albuminuria were, respectively, 1.9 (95% CI 0.9-4.1) and 1.3 (95% CI 0.6-2.8) after adjustment.

Table 3. Association between eGFR, albuminuria, and risk for venous thrombosis

	Adjusted* hazard ratios
eGFR	
> 90 ml/min	1.0 (reference)
60-90 ml/min	1.3 (0.7-2.3)
30-60 ml/min	1.5 (0.7-3.3)
	Adjusted† hazard ratios
Albuminuria‡	
No	1.0 (reference)
Yes	2.1 (1.4-3.2)

eGFR, estimated glomerular filtration rate. *Adjusted for age, sex, body mass index, hypertension, diabetes, malignancy, and hsCRP, and albuminuria (continuous). †Adjusted for age, sex, body mass index, hypertension, diabetes, malignancy, and hsCRP, and eGFR (continuous). ‡Albuminuria defined as urinary albumin excretion ≥30 mg per day

Table 4. Hazard ratios for venous thrombosis by decreased glomerular filtration rates and albuminuria

			Crude hazard ratios		Adjusted* hazard ratios	
			No albuminuria	Albuminuria†	No albuminuria	Albuminuria†
eGFR > 90 ml/min		HR (95% CI)	1.0 (reference)	4.8 (1.9-12.4)	1.0 (reference)	3.1 (1.1-8.9)
eGFR	60-90 ml/min	HR (95% CI)	2.2 (1.2-4.1)	5.2 (2.6-10.5)	1.5 (0.7-3.1)	2.7 (1.2-6.1)
eGFR	30-60 ml/min	HR (95% CI)	3.6 (1.4-9.3)	10.3 (4.2-24.7)	1.4 (0.5-4.1)	4.1 (1.5-11.0)

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; UAE, urinary albumin excretion per day. *Adjusted for age, sex, body mass index, hypertension, diabetes, malignancy, and hsCRP. †Albuminuria defined as urinary albumin excretion ≥30 mg per day

DISCUSSION

In this study including 8 495 subjects followed for over 8 years, we found a 2.2-fold (95% CI 0.9 -5.1) increased risk of VT in patients with CKD stage 1 and a 1.9-fold (95% CI 1.1 -3.1) increased risk of VT in patients with CKD stage 2 as compared with subjects without CKD according to the K/DOQI guidelines. CKD stage 3 patients with albuminuria had 3.0-fold (95% CI 1.4-6.5) increased risk of VT, and CKD stage 3 patients without albuminuria had a HR of 1.0 (95% CI 0.4-2.4). The risk of VT associated with CKD seemed to be related to albuminuria rather than to impaired eGFR. Furthermore, our findings showed that CKD stages 1-3 were mainly associated with unprovoked VT. Using the CKD-EPI formula instead of the MDRD formula for staging CKD did not result in large differences for any of the analyses.

Previous studies have investigated the association between eGFR on the basis of MDRD and VT.[10,16] In the study of the LITE cohort, HRs for VT were 1.3 (95% CI 1.0-1.6) for subjects with eGFR between 60 and 90 ml/min and 2.1 (95% CI 1.5-3.0) for subjects with eGFR between 15 and 60 ml/min (CKD stage 3-4) as compared with subjects with eGFR >90 ml/min.[10] However, information on albuminuria was not available in this study and formal classification into CKD stages was therefore not possible. In our study, we found a HR of 1.5 for VT for CKD stage 3 after adjustment for age, sex, and BMI; we showed that the risk of VT was only increased in the presence of albuminuria. Recent findings from the LITE study group contrast their earlier findings: eGFR based on cystatin was associated with an approximately 1.6-fold increased risk of VTE, while eGFR based on creatinine was not associated with an increased risk of VT.[16] The authors could not explain the discrepancy between the earlier and the current finding. Furthermore, albuminuria was not a risk factor for VT in their study, in contrast to our study. An explanation for this discrepancy could be that the relatively low prevalence of albuminuria may have limited their power to detect an association between VT and albuminuria, whereas our cohort was enriched for the presence of albuminuria. Moreover, in our study albuminuria was assessed in 24-h urine samples (gold standard) that were not frozen before assessment, whereas in their study albuminuria was assessed by albumin-creatinine ratio in frozen samples. Frozen storage is known to induce a systematic decrease and more variability in albuminuria concentration.[17] Furthermore, subjects with albuminuria are probably mainly diabetics in their study, while in PREVEND these are mainly non-diabetics, as per protocol insulin-using diabetic patients were excluded. This may have influenced the risk estimates, as diabetic subjects are usually on statin therapy and more frequently treated with anti-platelet medication for their cardiovascular morbidity. New findings indicate that statin use may reduce the risk of VT.[18]

Although the seemingly higher risk of VT in CKD stages 1 and 2 as compared with stage 3 might be surprising, the same pattern in the association between CKD and cardiovascular disease was previously found in the PREVEND study.[4] Patients with CKD stages 1 and 2 were at higher risk of cardiovascular disease than those with CKD stage 3. A plausible explanation for this might be the difference in staging of CKD stage 3 and CKD stages 1 and 2. Albuminuria is necessary to define CKD stages 1 and 2, whereas only GFR is needed to define CKD stage 3 to 5. Therefore, CKD stage 3 is a heterogeneous category, with subjects with and without evident kidney damage (albuminuria). We found that CKD stage 3 patients with albuminuria were at higher risk of VT than CKD stage 3 patients without albuminuria. These findings are in line with several other studies suggesting a higher risk for CKD stage 3 subjects with albuminuria than for CKD stage 3 subjects without albuminuria for different adverse outcomes, such as cardiovascular disease and the development of end-stage renal disease.[19-21] Taken together, these data suggest that information on albuminuria could be added to CKD stage 3 in order to improve the value of CKD staging for risk prognosis.

There are several possible mechanisms for the increased risk of VT in CKD. First, endothelial damage could explain the increased risk of VT. It is remarkable that the association between CKD stages 1-3 and VT was comparable with the previously reported association between CKD stages 1-3 and cardiovascular disease in the PREVEND study.[4] Therefore, it is tempting to hypothesize that a common risk factor for CKD leads to both VT and arterial cardiovascular disease. In our analysis, hypertension, BMI, and diabetes did not explain the increased risk of VT. Second, the increased risk of VT could be attributable to procoagulant changes in CKD patients which may be predominantly present in subgroups of CKD patients such as patients with nephrotic syndrome.[22] CKD and nephrotic syndrome have been associated with elevated levels of D-dimer, CRP, fibrinogen, factor VII, factor VIII, and von Willebrand factor,[23,24] which are important proteins in the development of VT. Third, inflammation may explain the increased risk of VT in CKD. It has been suggested that inflammation leads to VT.[25] However, additional adjustment of the HRs for hsCRP, which is currently the most widely used biomarker of inflammation,[26] did not alter the HRs in our study.

This study has several limitations. First, the K/DOQI guidelines require impaired GFR or albuminuria for at least three months. As in most studies, repeated measurements for a period of at least three months were not available in our study, and some subjects may therefore have been falsely classified as having CKD. Second, VT events were identified through anticoagulation clinic databases and registries for hospital discharge diagnoses and death certificates, which could lead to an underestimation of the incidence rates of VT. However, the incidence rates for VT in the PREVEND cohort (i.e. 1.4 per 1000 person-years) correspond well to those found in studies that had a complete case-finding procedure of objectively confirmed VT events.[27] Third, we may have underestimated renal function in subjects with a GFR >60 ml/min, because we used the MDRD study equation.[28,29] However, use of the CKD-EPI formula did not result in large differences in the HRs. Fourth, there are studies suggesting that risk of adverse events increases when GFR drops below 45 ml/min.[7,20] Our study did not include enough subjects with an eGFR <45 ml/min (n=52) to investigate this. Despite these limitations, PREVEND is a unique cohort in its large population-based prospective setting in which albuminuria was assessed in two 24-h urine samples.

We showed that CKD stages 1, 2, and 3 in the presence of albuminuria are risk factors for VT. The relative risk of VT for those with CKD stage 1-3 was 1.8-fold increased relative to those without CKD. Although these relative risk estimates may be considered to be weak as compared with, for example, relative risk estimates for venous thrombosis that have been reported for genetic thrombophilia,[30] on a population level CKD may be an important contributor to VT, because of the high prevalence of CKD, i.e. 12.7% for CKD stages 1-3 in the general population.[3] This is greater than most well-known genetic risk factors for VT, such as

prothrombin gene mutation.[31] Clinicians should be aware of the increased risk of VT in these patients. Further studies are needed to show whether VT prophylaxis in subgroups of these patients will be safe and cost-effective, especially as the high risk of anticoagulant treatment-related major bleeding episodes applies to CKD stage 4 and 5, and not CKD stage 1-3.[32]

In conclusion, CKD stages 1 and 2, and CKD stage 3 in presence of albuminuria were risk factors for VT. The risk of VT is more related to albuminuria than to impaired eGFR.

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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.

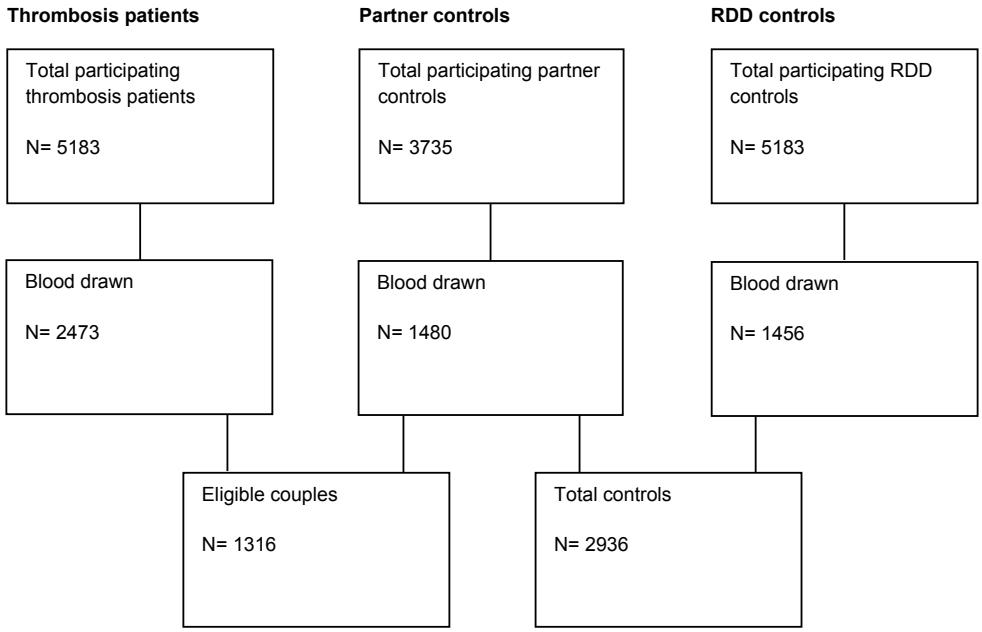

Figure 1. Flow chart of the MEGA study

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.

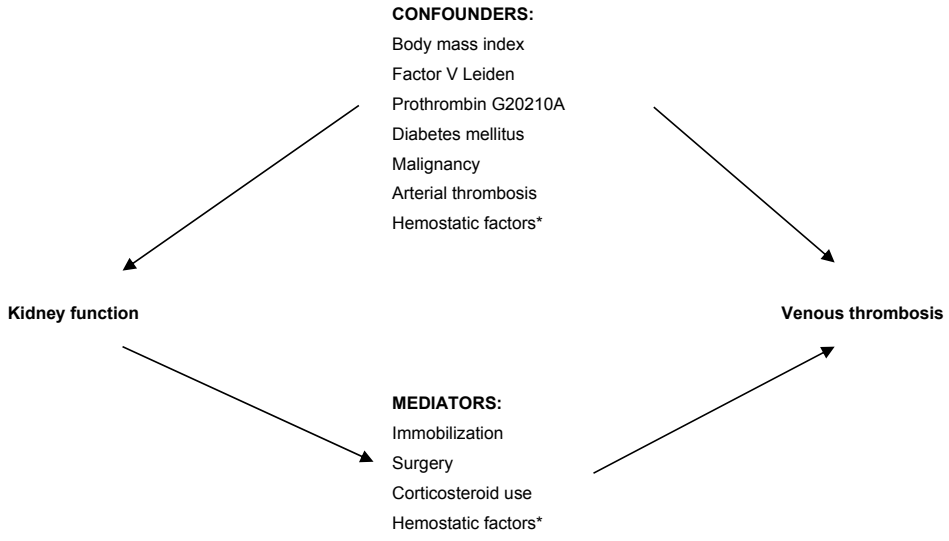

Figure 2. Causal diagram on the association between kidney function and venous thrombosis
*Hemostatic factors could be both confounders and mediators in the association between kidney function and venous thrombosis

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 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.

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
	10th - 50th percentile (eGFR 68-86 ml/min) N=1175	5th - 10th percentile (eGFR 64-68 ml/min) N=147	2.5th - 5th percentile (eGFR 59-64 ml/min) N=74	1st – 2.5th percentile (eGFR 53-64 ml/min) N=43	<1st 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 3. Effect of mildly and moderately to severely decreased kidney function on hemostatic factors in control subjects*

Hemostatic factor	Adjusted† mean difference (95% confidence interval) as compared with normal kidney function (eGFR >90 ml/min)					
	Pooled controls		Partner controls		RDD controls	
	eGFR 60-90 ml/min N=1672	eGFR <60 ml/min N=85	eGFR 60-90 ml/min N=836	eGFR <60 ml/min N=41	eGFR 60-90 ml/min N=836	eGFR <60 ml/min N=44
Anticoagulant factors						
Protein S*, IU/dL	0 (-1 to 2)	4 (0 to 9)	0 (-3 to 2)	3 (-4 to 10)	1 (-1 to 3)	6 (0 to 12)
Protein C*, IU/dL	0 (-2 to 1)	0 (-5 to 5)	-2 (-4 to 0)	-5 (-12 to 2)	2 (0 to 4)	6 (-1 to 12)
Antithrombin, IU/dL	0 (-1 to 1)	1 (-1 to 4)	-1 (-2 to 1)	2 (-1 to 6)	-1 (-1 to 2)	1 (-2 to 5)
Procoagulant factors						
Fibrinogen, g/L	-0.1 (-0.1 to 0.0)	0.3 (0.1 to 0.4)	-0.1 (-0.1 to 0.0)	0.3 (0.1 to 0.5)	-0.1 (-0.1 to 0.0)	0.2 (0.0 to 0.4)
Factor II*, IU/dL	1 (-1 to 2)	0 (-4 to 4)	0 (-2 to 1)	-3 (-9 to 4)	2 (-2 to 7)	2 (0 to 3)
Factor VII*, IU/dL	1 (-1 to 3)	10 (5 to 16)	1 (-2 to 3)	6 (-2 to 14)	1 (-1 to 4)	15 (8 to 23)
Factor VIII, IU/dL	6 (2 to 9)	41 (31 to 51)	6 (1 to 11)	39 (24 to 54)	5 (0 to 9)	42 (28 to 55)
VWF, IU/dL	3 (-1 to 6)	32 (21 to 43)	3 (-2 to 8)	35 (19 to 52)	2 (-3 to 7)	29 (15 to 43)
Factor IX*, IU/dL	-1 (-3 to 1)	3 (-1 to 8)	-2 (-4 to 0)	0 (-7 to 6)	8 (2 to 13)	0 (-2 to 2)
Factor X*, IU/dL	0 (-2 to 1)	1 (-4 to 5)	-1 (-4 to 1)	-3 (-9 to 4)	1 (-1 to 3)	5 (-2 to 11)
Factor XI, IU/dL	2 (0 to 3)	7 (3 to 11)	1 (-1 to 3)	11 (5 to 18)	2 (0 to 4)	4 (-2 to 10)

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

Table 4. Kidney function and risk of venous thrombosis

				ODDS RATIOS (95% CI)				
Percentiles	**eGFR (ml/min)**	Thrombosis Patients N	Controls N	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
>50th	**>86**	1165	1468	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
10th - 50th	**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)
5th - 10th	**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)
2.5th – 5th	**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)
1st - 2.5th	**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)
<1st	**<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)

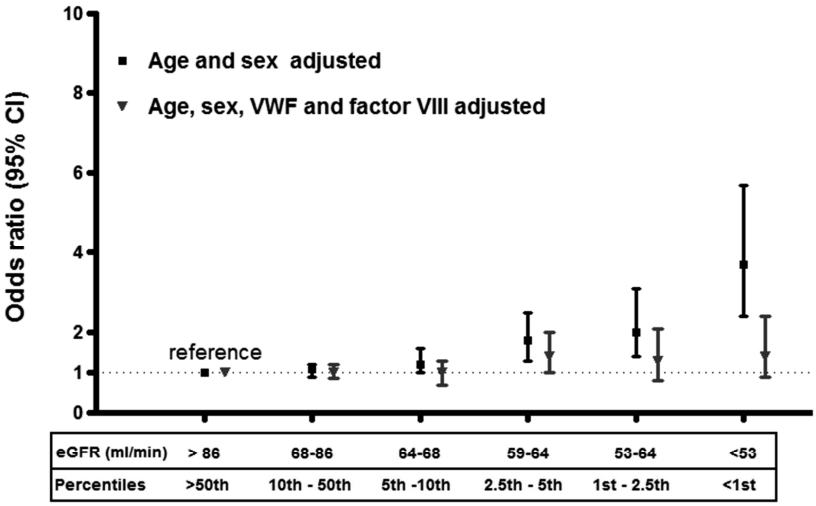

Figure 3. Percentiles of kidney function and risk of venous thrombosis

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** N (%)	**Pooled controls** N (%)	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
>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)
	Thrombosis Patients	**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)
	Thrombosis Patients	**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)

			ODDS RATIOS (95% CI)				
eGFR (ml/min)	Thrombosis Patients N (%)	Controls N (%)	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
>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 to12 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 factor[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.[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 to12 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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Chapter 5

Risk of venous thrombosis in patients with chronic kidney disease: Identification of high-risk groups

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ABSTRACT

Background: Although an association between venous thrombosis and chronic kidney disease has recently been established, it is unknown which patients with chronic kidney disease are most likely to benefit from thromboprophylaxis. The aim of this study was to assess the association between venous thrombosis and chronic kidney disease in combination with arterial thrombosis, malignancy, surgery, and thrombophilia to identify high-risk groups as a basis for personalized prevention.

Methods: This study included 2473 consecutive patients with first venous thrombosis and 2936 controls from a case-control study (the MEGA study).

Results: Moderately decreased kidney function (eGFR 30-60 ml/min) was associated with a 2.5-fold (95%CI 1.9-3.4) increased risk and severely decreased kidney function (eGFR <30 ml/min) was associated with a 5.5-fold (95%CI 1.8-16.7) increased risk, compared with those with normal kidney function (eGFR >90 ml/min). The risk of venous thrombosis was additionally increased for moderately and severely reduced kidney function in combination with arterial thrombosis (odds ratio 4.9; 95%CI 2.2-10.9), malignancy (5.8; 95%CI 2.8-12.1), surgery (14.0; 95%CI 5.0-39.4), immobilization (17.1; 95%CI 6.8-43.0), or thrombophilia (odds ratios 4.3-9.5), with particularly high risks when three or more risk factors were present (odds ratio 56.3; 95% CI 7.6-419.3).

Conclusion: Decreased kidney function is associated with an increased risk of venous thrombosis. The risk increased substantially in the presence of one or more other risk factors for thrombosis.

INTRODUCTION

Chronic kidney disease is an established risk factor for arterial thrombosis.[1-3] Until recently, it has been unclear whether chronic kidney diseases also increase the risk of venous thrombosis. In the Longitudinal Investigation of Thromboembolism Etiology (LITE) study, chronic kidney disease was associated with venous thrombosis in individuals older than 45 years of age.[4] This study reported that individuals with an estimated glomerular filtration rate between 15-60 ml/min had a 2.1-fold increased risk of venous thrombosis as compared with those with a normal kidney function. Recently, we confirmed these results in the Prevention of Renal and Vascular Disease (PREVEND) cohort study.[5] The moderately increased risk found in the above studies for chronic kidney disease probably does not justify the use of thromboprophylaxis when weighed against the thromboprophylaxis-related risk of major bleeding episodes.[6] Having chronic kidney disease in combination with other risk factors or high risk situations, such as immobilization, the presence of prothrombotic genes or other comorbidities, might increase the risk to such an extent that thromboprophylaxis would be recommendable. However, no studies have reported on the risk of venous thrombosis in persons with such combinations of risk factors.

We therefore calculated the estimated glomerular filtration rate in 2473 patients with a recent venous thrombosis and 2936 control subjects participating in a case-control (MEGA) study. The size of the study enabled us to investigate the risk for various glomerular filtration rates as well as the effects of combination with one or more other risk factors for thrombosis, with the aim of identifying high-risk groups that may benefit from thromboprophylaxis.

METHODS

Study design

The MEGA study (Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis study) is a large 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 (leg or arm) 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.[7]

Patients

Only patients with a diagnosis of venous thrombosis that was confirmed with objective techniques were included in the analyses, as previously described.[7] Exclusion criteria were severe psychiatric problems and inability to speak Dutch. Of the 6567 eligible patients, 5184

participated (79%). For logistic reasons, blood sampling was performed for patients included up to June 2002.Therefore, in the current analyses 2473 patients out of the 5184 patients (48%) who had their blood drawn were included.

Controls

As control individuals, partners of patients aged <70 years without venous thrombosis were included, as well as individuals without venous thrombosis obtained via a random-digit-dialing (RDD) method. Of the 5184 participating patients, 3735 had an eligible partner. Of the 3735 eligible partners, 3039 participated (81%). The RDD control individuals were recruited from the same geographical area as the patients, and were frequency matched to the patients on age and sex. Of the 4350 eligible random controls, 2789 participated (64%). This resulted in a total of 5828 control individuals without venous thrombosis. Blood was drawn in participants included until June 2002, again for the same logistic reasons, which included 2936 control individuals (50%).

Data collection

All individuals were asked to complete an extensive questionnaire on many potential risk factors for venous thrombosis. Of particular interest for the current analysis are items on demographics (including age and sex), immobilization (defined as being bedridden for more than 4 days or hospitalization within 3 months prior to the index date), surgery within 3 months prior to the index date, history of arterial thrombosis (myocardial infarction, angina pectoris, ischemic stroke, transient ischemic attack, and peripheral vascular disease) (self-reported) and malignancy (validated for participating patients with cancer by reviewing discharge letters from their primary physician or from the hospital in which they were being treated).[8] 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. All assays were performed in an automated machine by laboratory technicians who were unaware of the case-control status of the samples. 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.[9] Common genetic risk factors were assessed, including the factor V Leiden mutation and the prothrombin G20210A mutation, by polymerase chain reactions using the TaqMan assay.

Statistical analysis

To determine whether chronic kidney disease was associated with an increased risk for venous thrombosis, odds ratios (ORs) with 95% confidence intervals (95% CIs) adjusted for age and sex were calculated using unconditional logistic regression as estimates of the relative risk for mildly decreased kidney function (glomerular filtration rate 60-90 ml/min), moderately decreased kidney function (glomerular filtration rate 30-60 ml/min), and severely decreased kidney function (glomerular filtration rate <30 ml/min), as compared with normal kidney function. In our study, partner controls were matched on time to cases and random controls were selected from a stable, dynamic population.[10] We also investigated the association with venous thrombosis for glomerular filtration rate categories based on percentiles instead of using these clinical cut-off points for kidney function. In addition, odds ratios for venous thrombosis were calculated in order to identify high-risk groups, combining moderately to severely decreased kidney function (estimated glomerular filtration rate of <60 ml/min) with a priori specified risk factors (i.e. arterial thrombosis, malignancy, surgery, immobilization), and thrombophilia (factor V Leiden and prothrombin G20210A). As we were primarily interested in establishing high-risk groups that may benefit from thromboprophylaxis, we only adjusted for the matching factors, i.e. age and sex.[11,12] Adjusting for other factors when determining the association between chronic kidney disease and venous thrombosis is only of interest when determining the causal relationship. However, from a prediction point of view it is preferable to only determine the association between the predictive marker (chronic kidney disease) and the outcome (venous thrombosis), because adjustment could lead to false conclusions with respect to identifying high-risk groups. Statistical analyses were performed with statistical package SPSS Windows version 17.0 (SPSS Inc., Chicago, IL).

RESULTS

Table 1 shows the baseline characteristics of the study population. 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. Venous thrombosis patients had, as expected, more often arterial thrombosis, malignancy, surgery, immobilization, factor V Leiden mutation, or prothrombin G20210A than controls.

Table 1. Baseline characteristics

	Thrombosis patients N=2473		Controls N=2936	
Median age, years (5-95th %)	49.1	(25.9-67.5)	49.8	(27.1-67.0)
Women, n (%)	1346	(54.4%)	1543	(52.6%)
Arterial thrombosis, n (%)	147	(6.7%)	133	(4.8%)
Malignancy, n (%)	201	(8.2%)	107	(3.7%)
Surgery, n (%)	413	(16.7%)	85	(2.9%)
Immobilization, n (%)	663	(27.0%)	170	(5.8%)
Factor V Leiden, n (%)	390	(15.8%)	145	(4.9%)
Prothrombin G20210A, n (%)	117	(4.7%)	52	(1.8%)
Type of venous thrombosis, n (%)				
Deep vein thrombosis only	1473	(59.6%)		
Pulmonary embolism	1000	(40.4%)		

No major differences in mean glomerular filtration rates were observed when patients were tested on glomerular filtration rate 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, suggesting that glomerular filtration rates were not influenced by a temporarily raised effect.

As shown in Table 2, the age- and sex-adjusted odds ratios of venous thrombosis increased with increasing severity of kidney disease compared with normal kidney function (glomerular filtration rate >90 ml/min): the odds ratio was 1.1 (95% CI 1.0-1.2) for mildly decreased kidney function (estimated glomerular filtration rate 60-90 ml/min), 2.5 (95% CI 1.9-3.4) for moderately decreased kidney function (estimated glomerular filtration rate 30-60 ml/min), and 5.5 (95% CI 1.8-16.7) for severely decreased kidney function (estimated glomerular filtration rate <30 ml/min). The odds ratios were similar for deep vein thrombosis and pulmonary embolism as separate outcomes.

When defining glomerular filtration rate categories based on percentiles instead of clinical cut-off points for kidney function, we also found, beside an increase in venous thrombosis risk with decreasing estimated glomerular filtration rate (Figure 1), an increased risk of venous thrombosis (odds ratio 1.4; 95% CI 1.0-1.9) for participants with glomerular hyperfiltration (i.e. those with the highest 2.5 percentile of the kidney function, corresponding to an estimated glomerular filtration rate of more than 125 ml/min).

To investigate joint effects of reduced kidney function and common risk factors for venous thrombosis, we categorized kidney function into two groups: reduced kidney function (moderately to severely decreased; estimated glomerular filtration rate <60 ml/min) and normal kidney function (estimated glomerular filtration rate 60-125 ml/min). Subjects with estimated

glomerular filtration rate of more than 125 ml/min were excluded from this analysis because their risk of venous thrombosis was also increased. Surgery in the absence of reduced kidney function increased the risk of venous thrombosis 6.9-fold (95% CI 5.3-8.8), while surgery combined with reduced kidney function increased the risk of venous thrombosis 14-fold (95% CI 5.0-39.4) (Table 3). Immobilization in the absence of reduced kidney function increased the risk of venous thrombosis 5.7-fold (95% CI 4.8-6.9), while immobilization combined with reduced kidney function increased the risk of venous thrombosis 17.1-fold (95% CI 6.8-43.0) (Table 3). The presence of all other risk factors, including malignancy, factor V Leiden mutation, or prothrombin G20210A, also additionally increased the risk for patients with reduced kidney function (Table 3).

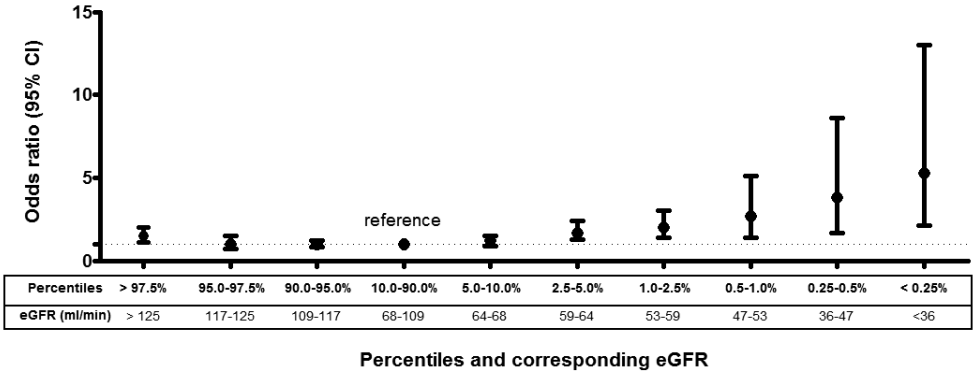

Figure 1. Percentiles of kidney function and risk of venous thrombosis

The risk of venous thrombosis further increased when more than one risk factor was present (Table 4). In the absence of immobilization, surgery, malignancy, arterial thrombosis, factor V Leiden, and prothrombin gene mutation, a reduced estimated glomerular filtration rate <60 ml/min was still associated with a two-fold increased risk of venous thrombosis (odds ratio 2.0, 95% CI 1.3-3.0). The presence of three or more of the six risk factors for venous thrombosis was associated with 56.3-fold (95% 7.6-419.3) increased risk of venous thrombosis.

There was a high risk of venous thrombosis for patients with chronic kidney disease patients with concomitant arterial thrombosis with surgery (odds ratio 17.9; 95% CI 2.2-146.2) or immobilization (odds ratio 16.8; 95% CI 3.8-75.1). In addition, chronic kidney disease patients with concomitant malignancy in combination with immobilization had a high risk of venous thrombosis (odds ratio 52.3; 95% CI 7.0-390.4). Also, the combination of chronic kidney disease with prothrombin G20210A or factor V Leiden in the presence of immobilization resulted in a high risk of venous thrombosis (odds ratio 17.8; 95% CI 4.0-78.7).

Table 2. Association between chronic kidney disease and venous thrombosis

eGFR (ml/min)	Description kidney function	Thrombosis Patients N	(%)	Controls N	(%)	Adjusted* odds ratio for venous thrombosis	Adjusted*† odds ratio for DVT	Adjusted*‡ odds ratio for PE
eGFR > 90	Normal	937	(37.9)	1179	(40.2)	1 (reference)	1 (reference)	1 (reference)
eGFR 60- 90	Mildly decreased	1376	(55.6)	1672	(56.9)	1.1 (1.0-1.2)	1.1 (0.9-1.2)	1.1 (1.0-1.3)
eGFR 30-60	Moderately decreased	144	(5.8)	81	(2.8)	2.5 (1.9-3.4)	2.2 (1.6-3.1)	2.9 (2.0-4.2)
eGFR < 30	Severely decreased	16	(0.6)	4	(0.1)	5.5 (1.8-16.7)	7.0 (2.2-21.8)	3.4 (0.8-13.7)

*Adjusted for age and sex; †DVT, deep vein thrombosis only; ‡PE, pulmonary embolism with or without deep vein thrombosis

Table 3. Joint effects on venous thrombosis of chronic kidney disease and surgery, immobilization, malignancy, factor V Leiden, and prothrombin G20210A

Decreased kidney function*	Acquired or genetic risk factor	Thrombosis patients		Controls		Adjusted† odds ratio (95% CI)	
		N	(%)	N	(%)		
	Arterial thrombosis						
No	No	1878	(87.8)	2524	(92.4)	1	(reference)
Yes	No	114	(5.3)	75	(2.7)	2.2	(1.6-3.0)
No	Yes	120	(5.6)	124	(4.5)	1.4	(1.1-1.8)
Yes	Yes	26	(1.2)	8	(0.3)	4.9	(2.2-10.9)
	Malignancy						
No	No	2053	(86.6)	2674	(93.6)	1	(reference)
Yes	No	124	(5.2)	76	(2.7)	2.3	(1.7-3.1)
No	Yes	159	(6.7)	98	(3.4)	2.3	(1.8-3.0)
Yes	Yes	36	(1.5)	9	(0.3)	5.8	(2.8-12.1)
	Surgery						
No	No	1845	(72.8)	2681	(94.3)	1	(reference)
Yes	No	124	(4.4)	79	(2.8)	2.4	(1.8-3.3)
No	Yes	366	(20.6)	78	(2.7)	6.9	(5.3-8.8)
Yes	Yes	36	(2.2)	4	(0.1)	14.0	(5.0-39.4)
	Immobilization						
No	No	1633	(68.9)	2600	(91.5)	1	(reference)
Yes	No	107	(4.5)	76	(2.7)	2.3	(1.7-3.2)
No	Yes	579	(24.4)	160	(5.6)	5.7	(4.8-6.9)
Yes	Yes	52	(2.2)	5	(0.2)	17.1	(6.8-43.0)
	Factor V Leiden						
No	No	1871	(78.4)	2642	(92.3)	1	(reference)
Yes	No	136	(5.7)	77	(2.7)	2.6	(2.0-3.5)
No	Yes	358	(15.0)	134	(4.7)	3.8	(3.1-4.6)
Yes	Yes	23	(1.0)	8	(0.3)	4.3	(1.9-9.7)
	Prothrombin G20210A						
No	No	2120	(88.8)	2726	(95.3)	1	(reference)
Yes	No	152	(6.4)	84	(2.9)	2.5	(1.9-3.3)
No	Yes	109	(4.6)	50	(1.7)	2.8	(2.0-3.9)
Yes	Yes	7	(0.3)	1	(0.0)	9.5	(1.2-77.4)

*Decreased kidney function defined as eGFR<60 ml/min as compared with eGFR 60-125 ml/min; †Adjusted for age and sex

Table 4. Odds ratios for venous thrombosis for total number of risk factors present per person

Chronic kidney disease*	Number of genetic/acquired risk factors†	Age and sex adjusted odds ratio (95% CI)
No	0	1 (reference)
Yes	0	2.0 (1.3-3.0)
No	1	3.2 (2.8-3.7)
Yes	1	7.8 (4.4-13.8)
No	2	9.6 (7.3-12.5)
Yes	2	7.6 (3.0-19.2)
No	≥ 3	13.2 (7.7-22.6)
Yes	≥ 3	56.3 (7.6-419.3)

*Chronic kidney disease eGFR<60ml/min (Yes) as compared with eGFR 60-125 ml/min; †Risk factors: arterial thrombosis, malignancy, surgery, immobilization, factor V Leiden, or prothrombin G20210A

DISCUSSION

In this large case-control study, kidney function showed an inverse association with venous thrombosis risk with a nearly 6-fold increased risk for those with severely decreased kidney function (estimated glomerular filtration rate <30 ml/min). Those with additional risk factors had an even higher risk of thrombosis, particularly patients who were immobilized or underwent surgery (around 15-fold increased risk). Furthermore, there was a cumulative effect when several risk factors were present simultaneously with renal function impairment, with up to 56-fold increased risks. Since these involve common medical circumstances, these findings may offer a tool for targeted thromboprophylaxis in vulnerable patients.

In line with our results, both the LITE[4] and PREVEND[5] study found an increased risk of venous thrombosis for chronic kidney disease. Interestingly, we showed that a high glomerular filtration rate of more than 125 ml/min was also associated with an increased risk of venous thrombosis (odds ratio 1.4; 95% CI 1.0-1.9). A high glomerular filtration rate has been shown to be an indicator for early kidney disease and a predictor of cardiovascular disease.[13-16]

Based on the odds ratios of venous thrombosis for decreased kidney function ranging from 1.1 for mildly decreased kidney function (estimated glomerular filtration rate 60-90 ml/min) to 5.5 for severely decreased kidney function (estimated glomerular filtration rate <30 ml/min), thromboprophylaxis is probably not justified in all patients with decreased kidney function since it does not seem to outweigh the increased bleeding risk associated with decreased kidney function.[17,18] However, our data imply that chronic kidney disease is especially relevant when it is present in combination with other risk factors for venous thrombosis, because we found a 4- to 17-fold increased risk when chronic kidney disease was jointly present with arterial thrombosis, malignancy, surgery, immobilization, factor V Leiden, or prothrombin G20210A.

The risk of venous thrombosis was 56.3-fold increased in the presence of chronic kidney disease and three or more of the six risk factors (arterial thrombosis, malignancy, surgery, immobilization, factor V Leiden, and prothrombin G20210A). These observations could have clinical implications. The American College of Chest Physicians (ACCP) currently recommends pharmacologic thromboprophylaxis for several hospitalized groups at high risk for venous thrombosis, including patients with a major trauma, spinal cord injury, or heart failure,[6] but not including patients with chronic kidney disease. Our study results suggest, however, that these patients are also at increased risk of venous thrombosis, especially in combination with one or more other risk factors. In patients with arterial thrombosis or malignancy who are immobilized or will undergo surgery, creatinine measurements are often routinely performed (for example before diagnostic testing with contrast agents, for medication dosing, or to identify concurrent kidney damage) and can therefore be easily taken into account when deciding on thrombosis prophylaxis. However, a potential problem in patients with a chronic kidney disease is the increased bleeding risk associated with anticoagulation use.[17,18] Therefore, each person's risks and benefits need to be weighed individually until there are randomized clinical trials answering these questions. Furthermore, screening for thrombophilia in patients with chronic kidney disease is probably not justified given the low prevalence of thrombophilia (factor V Leiden and prothrombin gene mutation) and given the moderately increased risks of venous thrombosis.

Decreased glomerular filtration has been associated with endothelial dysfunction and subsequent arterial thrombosis.[1-3,19] Endothelial dysfunction has also been associated with changes in the levels of several coagulation proteins and with an increased venous thrombosis risk.[20] Hence, in theory, the association between chronic kidney disease and venous thrombosis could be causally explained through endothelial dysfunction. However, these assumptions are merely based on literature,[21] and we did not assess endothelial dysfunction in this study. Addressing the mechanism through which chronic kidney disease increases the risk of venous thrombosis was not the aim of this study, as we were primarily interested in establishing high-risk groups that may benefit from thromboprophylaxis, irrespective of the underlying causal relation. Research into this relation has yet to be conducted.

A limitation of this study is that we had no information about proteinuria. It would be useful to explore whether proteinuria in combination with decreased kidney function is associated with a more increased risk of venous thrombosis than decreased kidney function alone. Proteinuria, especially in the nephrotic range, has been associated with venous thrombosis.[22-24] Another limitation was that arterial thrombosis was self-reported. However, we expect that patients and control persons misreported arterial thrombosis similarly and infrequently, because these are major diseases with a large impact limiting recall bias. Random misclassification would

result in an underestimation of our odds ratios. Of note, we used the cut-off levels for the glomerular filtration rate of the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) guidelines to define kidney disease.[25] Nevertheless, it would have been interesting to identify risk of venous thrombosis in, for example, kidney-transplanted patients or patients who had hemodialysis or peritoneal dialysis treatment, but this information was not available in our study. Strengths of this study include the large patient sample and detailed information about other risk factors for venous thrombosis such as history of arterial thrombosis, malignancy, surgery, immobilization, and genetic risk factors. Nevertheless in some subgroups numbers became small, which may have led to slightly inflated risk estimates.

In summary, in a large case-control population we found that a decreasing kidney function was associated with an increasing risk of venous thrombosis. The risk of venous thrombosis in individuals with chronic kidney disease was further increased in the presence of additional risk factors for venous thrombosis. These high-risk groups could be considered for future intervention trials into the effectiveness of thromboprophylaxis.

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Chapter 6

Venous and arterial thrombosis in dialysis patients

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ABSTRACT

Background: Whether the risk of both venous and arterial thrombosis is increased in dialysis patients as compared to the general population is unknown. In addition, it is unknown which subgroups are at highest risk. Furthermore, it is unknown whether having a history of venous thrombosis or arterial thrombosis prior to dialysis treatment increases mortality risk.

Methods: A total of 455 dialysis patients were followed for objectively verified symptomatic thrombotic events between January 1997 and June 2009.

Results: The incidence rates in dialysis patients as compared to the general population was 5.6-fold (95% CI 3.1-8.9) increased for venous thrombosis, 11.9-fold (95% CI 9.3-14.9) increased for myocardial infarction, and 8.4-fold (95% CI 5.7-11.5) increased for ischemic stroke. The combination of hemodialysis, lowest tertile of albumin, history of venous thrombosis, and malignancy was associated with subsequent venous thrombosis. Increased age, renal vascular disease, diabetes, high cholesterol levels, history of venous thrombosis, and history of arterial thrombosis were associated with subsequent arterial thrombosis. The all-cause mortality risk was 1.9-fold (95% CI 1.1-3.3) increased for patients with a history of venous thrombosis and 1.9-fold (95% CI 1.4-2.6) increased for patients with a history of arterial thrombosis. A potential limitation of this study was that in some risk categories associations with venous thrombosis did not reach statistical significance due to small numbers.

Conclusion: Dialysis patients have clearly elevated risks of venous thrombosis and arterial thrombosis and occurrence of venous thrombosis or arterial thrombosis prior to the start of dialysis is associated with an increased mortality risk.

INTRODUCTION

In the past, venous and arterial thrombosis have been regarded as separate diseases with different causes.[1] In the last decade, however, several investigators suggested that venous and arterial thrombosis might not be fully separate entities as several studies have shown that patients with venous thrombosis have an increased risk of arterial thrombosis and vice versa.[2-6] Additional studies have shown that arterial and venous thrombosis share some risk factors, although this has only consistently been shown for obesity.[7-11]

Early stages of chronic kidney disease have been associated with both venous and arterial thrombosis.[12,13] However, end-stage renal disease has only been associated with arterial thrombosis,[14-20] and not with venous thrombosis including deep vein thrombosis and pulmonary embolism. One study in the US Renal Data System (USRDS) showed that dialysis patients had an age-adjusted 2.3-fold increased risk of for a primary discharge diagnosis of pulmonary embolism occurring within the first year of dialysis treatment as compared to the general population.[21] However, deep vein thrombosis was not assessed in this study.

Therefore, the primary aim of this study was to assess the absolute risk of deep vein thrombosis and pulmonary embolism (venous thrombosis) and myocardial infarction and ischemic stroke (arterial thrombosis) in a cohort of end-stage renal disease patients receiving dialysis treatment. We also assessed whether venous thrombosis and arterial thrombosis shared risk factors in dialysis patients. Finally, we determined whether having a history of venous and arterial thrombosis prior to start of dialysis treatment increased the mortality risk.

METHODS

Patients

The Netherlands Cooperative Study on the Adequacy of Dialysis (NECOSAD) is a prospective multicenter cohort study in which incident adult end-stage renal disease patients in the Netherlands were included. Eligibility included age older than 18 years, and no previous renal replacement therapy. All patients gave informed consent and the study was approved by all local medical ethics committees. We followed 455 patients, from January 1997 in three dialysis centers that participated in NECOSAD, until a thrombotic event (venous thrombosis, myocardial infarction, and ischemic stroke), death, or censoring, i.e. transfer to a nonparticipating dialysis center, withdrawal from the study, transplantation, or end of the follow-up period (June 2009). These three centers were chosen for logistic reasons, i.e. they provided a large number of patients.

Demographic and clinical data

Data on age, sex, primary kidney disease, smoking status, diabetes, medication, and history of thromboembolic events (venous thrombosis, myocardial infarction, or ischemic stroke) were collected at the start of dialysis treatment. Primary kidney disease was classified according to the codes of the European Renal Association-European Dialysis and Transplant Association (ERA-EDTA).[22] We grouped patients into four classes of primary kidney disease: glomerulonephritis, diabetes mellitus, renal vascular disease, and other kidney diseases. Other kidney diseases consisted of patients with interstitial nephritis, polycystic kidney diseases, other multisystem diseases and unknown diseases.

Serum albumin, hemoglobin, creatinine, urea, total cholesterol, and triglycerides were routinely measured in the dialysis centers at 3 months after start of dialysis. Total protein, urea, and creatinine levels were also routinely measured in 24-hour urine samples. Renal function, expressed as glomerular filtration rate (GFR), was calculated as the mean of creatinine and urea clearance corrected for body surface area (ml/min per 1.73 m^2).

Venous thrombosis and arterial thrombosis

Symptomatic venous thrombosis (deep vein thrombosis of the leg and pulmonary embolism) and symptomatic arterial thrombosis (myocardial infarction and ischemic stroke) during follow-up were identified from hospital diagnosis registration systems and from chart review of all 455 patients. Moreover, we used medical records to validate the thrombotic events. Peripheral vascular atherosclerotic diseases were not considered as arterial events due to lack of detailed information of these disease entities in our patients charts.

Venous thrombosis was considered confirmed when diagnosed by compression ultrasound for deep vein thrombosis of the leg and/or when diagnosed by spiral computed tomography or ventilation-perfusion lung scanning for pulmonary embolism. Venous thrombosis was considered unprovoked in the absence of surgery, trauma, presence of a catheter, immobilization for >7 days or hospitalization, oral contraceptives, hormone therapy, pregnancy, malignant disease, or long-distance travel for >4 hours at or within one month before the development of venous thrombosis. Medical records were reviewed with a standardized check-list to categorize venous thrombosis into provoked or unprovoked.

Myocardial infarction had to be confirmed by typical symptoms, electrocardiogram features, elevated levels of cardiac enzymes, radionuclide imaging techniques, or coronary angiography. Ischemic stroke had to be diagnosed by computed tomography or magnetic resonance imaging.

Mortality

We classified causes of death according to the codes of the European Renal Association-European Dialysis and Transplantation Association (ERA-EDTA) which is a standardized classification of death causes in dialysis patients.[22] We grouped death causes into cardiovascular and non-cardiovascular. Cardiovascular mortality was defined as death due to myocardial ischemia and infarction (code 11); cardiac arrest/ sudden death (code 15); cardiac failure/ fluid overload/ pulmonary edema (codes 14,16,18); hyperkalemia /hypokalemia (code 12,17); pulmonary embolism (code 21); cerebrovascular accident (code 22); hemorrhage from ruptured vascular aneurysm (code 26); mesenteric infarction (code 29); cause of death uncertain/unknown (code 0). Non-cardiovascular mortality was defined as death caused by pulmonary infection (code 31-33); infections elsewhere (code 34); septicemia (code 35); tuberculosis (code 36-37); generalized viral infection (code 38); peritonitis (code 39); suicide (code 52); treatment cessation (code 51, 53-54) ; cachexia (code 64) ; malignancies (codes 66-68); miscellaneous (codes 13, 23-28, 41-46, 61-63, 69-73, 81-82, 99-102).

Statistical analysis

Continuous variables are presented as mean with standard deviation (SD) or as median and interquartile rage (IQR) depending on the normality of the data. Categorical variables are presented as counts with corresponding percentages. The observation time for venous thrombosis in each participant was calculated as the time elapsed between the start of dialysis and a censoring event (withdrawal from the study, transplantation, death, or June 2009), or the first episode of venous thrombosis during dialysis. The observation time for arterial thrombosis in each participant was calculated as the time elapsed between the start of dialysis and a censoring event (withdrawal from the study, transplantation, death, or June 2009), or the first episode of arterial thrombosis during dialysis. Incidence rates for arterial and venous thrombosis were calculated by dividing the number of patients with a venous thrombosis or arterial thrombosis by the total observation time at risk. When calculating the incidence rates for venous thrombosis, we ignored the occurrence of arterial thrombosis and vice versa. Incidence rates and 95% confidence intervals (95% CIs) were calculated with Poisson regression models for venous thrombosis, myocardial infarction, and ischemic stroke in dialysis patients. We used indirect standardization to compare these incidence rates to the age- and sex-weighted incidence rates in the general population obtained from the HUNT2 study for venous thrombosis[23] and the Framingham study for myocardial infarction[24] and ischemic stroke.[25] The presented incidence rates in the general population are based on the age- and sex-distribution of the dialysis patients in our study. Cumulative incidences for venous thrombosis and arterial thrombosis were analyzed by using time-to-event analyses accounting for competing risk of transplantation and death.[26] Furthermore, we calculated adjusted hazard ratios (HRs) with 95% CIs to evaluate the effect of clinical and laboratory

characteristics on the development of venous thrombosis and arterial thrombosis. Finally, we determined whether having a history of venous and arterial thrombosis prior to start of dialysis treatment increased the (cardiovascular and non-cardiovascular) mortality. SPSS statistical software (version 17.0; SPSS, Chicago, Illinois) was used for the analyses.

RESULTS

Baseline characteristics of the 455 patients are shown in Table 1. Overall, the mean age was 60.4 years, 65.7% were male, 64.6% had hemodialysis treatment at initiation of dialysis including 85 patients with a catheter (18.7%) and 209 patients with an arteriovenous access (45.9%), and 18.2% of patients had diabetes as primary kidney disease. Of the 455 patients, 23 (5.1%) had a history of venous thrombosis and 116 (25.5%) had a history of arterial thrombosis prior to the start of dialysis therapy. Patients were followed for a median observation period of 2.4 years (range 0.1 to 11.7 years).

During the observation period, 15 patients developed venous thrombosis, of whom seven had pulmonary embolism, seven deep vein thrombosis, and one presented with both. Four patients (26.7%) with pulmonary embolism died. Of the 15 venous thrombotic events, 5 were unprovoked and 10 were provoked (hospitalization, n=4; catheter-related, n=4; surgery, n=2, presence of malignancy, n=2). Of the 4 patients who developed venous thrombosis during hospitalization, one had an exacerbation of ulcerative colitis, one patient had sepsis, one had a pancreatitis, and one had an exacerbation of Wegener's disease. Of the 4 patients who developed catheter associated venous thrombosis, three had a deep vein thrombosis and one had a pulmonary embolism. One patient developed venous thrombosis during hospitalization after coronary artery bypass grafting and another patient developed venous thrombosis shortly after thrombectomy of a thrombosed dialysis shunt. Of note, none of the patients had an arteriovenous access in the lower limb. Furthermore, 96 patients developed an arterial thrombosis (72 patients developed myocardial infarction of which 15 were fatal (20.8%) and 33 patients developed ischemic stroke of which 6 were fatal (18.2%).

Table 1. Baseline characteristics

	N=455	
Age, years	60.4	± 15.1
Sex		
Male	299	(65.7%)
Female	156	(34.3%)
Dialysis modality (%)		
Hemodialysis	294	(64.6%)
Peritoneal dialysis	161	(35.4%)
Primary kidney disease (%)		
Diabetes mellitus	83	(18.2%)
Glomerulonephritis	48	(10.5%)
Renal vascular disease	75	(16.5%)
Other	249	(54.7%)
Body mass index, kg/m^2	25.0	± 5.2
Diabetes mellitus as comorbidity	122	(26.8%)
Malignancy	24	(5.3%)
History of venous thrombosis	23	(5.1%)
History of arterial thrombosis	116	(25.5%)
Smoking		
Never	174	(40.7%)
Ever	254	(59.3%)
Hemoglobin, mmol/L	6.9	± 1.0
GFR, ml/min	3.3	(1.9-5.6)
Proteinuria, gram per day	1.1	(0.5-2.4)
Anticoagulation use	21	(6.7%)
Erythropoietin use	276	(60.7%)
Erythropoietin dose, IU/week	6000	(4000-8000)
Albumin, g/L	33.0	(29.0-37.0)
Cholesterol, mmol/L	4.4	(3.6-5.4)
Triglycerides, mmol/L	1.9	(1.3-2.6)

Figure 1 shows the incidence rates per 1000 person-years for venous thrombosis (combination of deep vein thrombosis and pulmonary embolism), deep vein thrombosis (alone), pulmonary embolism (with or without deep vein thrombosis), myocardial infarction, and ischemic stroke in dialysis patients as compared to the estimated age- and sex-weighted incidence rates in the general population (HUNT2 study[23] for venous thrombosis and Framingham study[24,25] for myocardial infarction and ischemic stroke). The incidence rate of venous thrombosis (12.3 (95% CI 7.2-19.9) per 1000 person-years) in dialysis patients was 5.6 (95% CI 3.1-8.9) times higher than the estimated age- and sex-weighted annual incidence rate in the general population (HUNT2 study,[23] 2.2 per 1000 person-years). The incidence of both provoked venous thrombosis (8.2 per 1000 person-years; 95% CI 4.2-14.6) and unprovoked venous thrombosis (4.0 per 1000 person-years; 95% CI 1.4-8.9) were higher than the age-

and sex-weighted annual incidence rates of provoked and unprovoked venous thrombosis in the general population (HUNT2 study,[23] 1.1 per 1000 person-years for provoked venous thrombosis and 1.1 per 1000 person-years for unprovoked venous thrombosis). The absolute risk of myocardial infarction (62.1 (95% CI 49.0-77.8) per 1000 person-years) was 11.9 (95% CI 9.3-14.9) times higher in dialysis patients than the estimated age- and sex-weighted incidence rate in the general population (the Framingham study,[24] 5.2 per 1000 person-years). Moreover, the absolute risk of ischemic stroke (27.6 (95% CI 19.3-38.4) per 1000 person-years) was 8.4 (95% CI 5.7-11.5) times higher in dialysis patients than the estimated age- and sex-weighted annual incidence rate in the general population (the Framingham study,[25] 3.3 per 1000 person-years). The cumulative incidence at eight years of follow-up was 4.1% for venous thrombosis and 24.8% for arterial thrombosis.

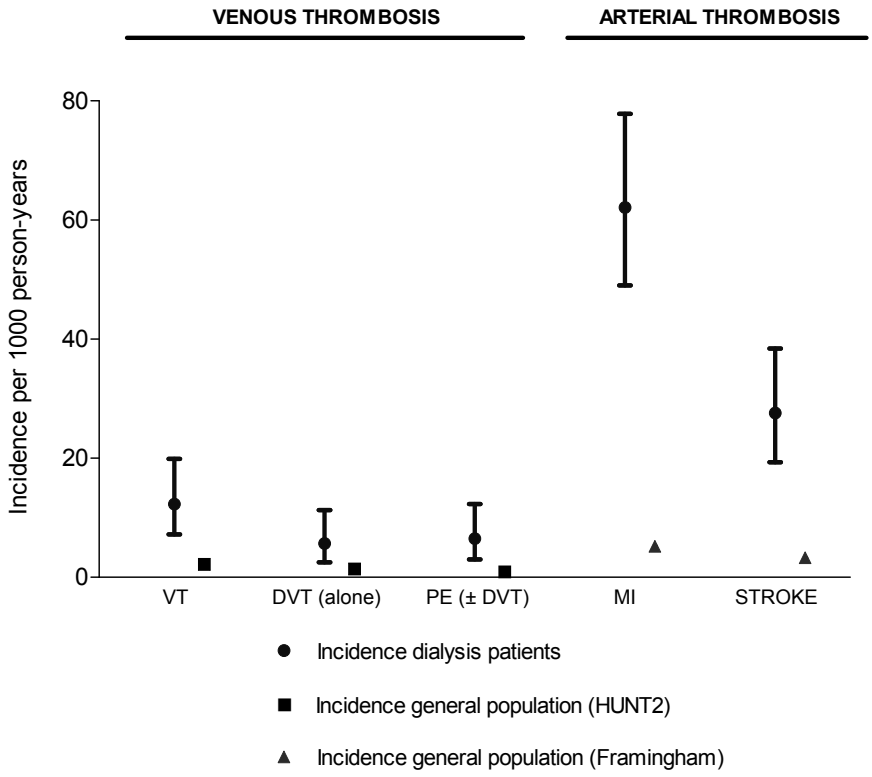

Figure 1. Incidence rates per 1000 person-years for venous and arterial thrombosis in dialysis patients as compared to the age- and sex- weighted incidence rates in the general population
MI indicates myocardial infarction; STROKE, ischemic stroke; VT, venous thrombosis; DVT, deep vein thrombosis; PE, pulmonary embolism

Table 2 shows the risk of venous or arterial thrombosis for different baseline variables after adjustment for age and sex. Venous and arterial thrombosis did not share risk factors in these dialysis patients, except for history of venous thrombosis which was associated with both venous and arterial thrombosis. Hemodialysis therapy, highest tertile of albumin, malignancy, and history of venous thrombosis were associated with venous thrombosis after adjustment for age and sex, although not significant. The combination of hemodialysis, highest tertile of albumin, history of venous thrombosis, and malignancy were associated with a 12.0-fold (95% CI 1.7-84.9) increased risk of venous thrombosis as compared with the absence of these risk factors. History of arterial thrombosis was not associated with subsequent venous thrombosis (hazard ratio 1.0; 95 CI 0.3-3.9). However, after exclusion of vitamin K antagonist users (anticoagulation use), the hazard ratio increased to 1.6 (95% CI 0.3-8.0). Increased age, diabetic nephropathy, renal vascular disease, history of arterial and venous thrombosis, diabetes as comorbidity, and the highest tertile of cholesterol were associated with arterial thrombosis. The combination of increased age (≥65 years), renal vascular disease, history of arterial and venous thrombosis, diabetes, and the highest tertile of cholesterol was associated with an 11.3-fold (95% CI 1.8-72.3) increased risk of arterial thrombosis as compared with the absence of these risk factors.

During the observation period, 197 patients died (99 cardiovascular mortality and 98 non-cardiovascular deaths). Patients with a history of venous or arterial thrombosis before starting dialysis had an increased mortality risk while on dialysis after adjustment for age, sex, diabetes, and primary kidney disease (Table 3): the all-cause mortality risk was 1.9-fold (95% CI 1.1-3.3) increased for patients with a history of venous thrombosis and 1.9-fold (95% CI 1.4-2.6) increased for patients with a history of arterial thrombosis as compared to patients without a history of venous or arterial thrombosis. Patients with a history of venous thrombosis had a non-significantly 2.0-fold (95% CI 0.9-4.4) increased risk of cardiovascular mortality and a non-significantly 1.8-fold (95% CI 0.8-4.0) increased risk for non-cardiovascular mortality. Patients with a history of arterial thrombosis had a 2.4-fold (95% CI 1.6-3.7) increased risk for cardiovascular mortality and a 1.5-fold (95% CI 1.0-2.4) increased risk for non-cardiovascular mortality.

Table 2. Association of baseline characteristics with subsequent venous and arterial thrombosis after adjustment for age and sex

		Venous thrombosis Hazard ratios* (95% CI)		Arterial thrombosis Hazard ratios* (95% CI)	
Age, years	< 65	1.0	(reference)	1.0	(reference)
	65-75	0.3	(0.1-1.6)	1.1	(0.7-1.7)
	>75	1.2	(0.3-4.5)	1.6	(1.0-2.8)
Sex	Male	0.8	(0.3-2.1)	1.3	(0.9-2.1)
	Female	1.0	(reference)	1.0	(reference)
Dialysis modality	Hemodialysis	2.6	(0.7-9.7)	0.7	(0.4-1.1)
	Peritoneal dialysis	1.0	(reference)	1.0	(reference)
Primary kidney disease	Diabetes mellitus	0.5	(0.1-2.2)	2.0	(1.2-3.4)
	Glomerulonephritis	0.4	(0.1-2.2)	1.2	(0.5-2.5)
	Renal vascular disease	NE		2.5	(1.5-4.2)
	Other	1.0	(reference)	1.0	(reference)
Body mass index, kg/m^2	<30.0	1.0	(reference)	1.0	(reference)
	≥30.0	1.6	(0.4-5.8)	0.8	(0.4-1.6)
Diabetes mellitus as comorbidity		1.5	(0.5-4.3)	1.5	(1.0-2.3)
Malignancy		3.0	(0.6-13.8)	0.6	(0.2-1.7)
History of venous thrombosis		3.4	(0.7-15.5)	2.3	(1.1-4.9)
History of arterial thrombosis		1.0	(0.3-3.9)	2.9	(1.9-4.5)
Smoking		1.2	(0.4-3.6)	1.5	(0.9-2.3)
Erythropoietin use		0.8	(0.3-2.3)	1.2	(0.8-1.8)
Hemoglobin, mmol/L	<6.5	1.0	(reference)	1.0	(reference)
	≥6.5 to 7.2	0.7	(0.2-2.3)	1.0	(0.6-1.7)
	>7.2	0.6	(0.2-2.0)	1.3	(0.8-2.1)
GFR, ml/min	0 to 5	1.0	(reference)	1.0	(reference)
	>5 to 10	0.9	(0.3-3.3)	1.0	(0.6-1.6)
	>10	1.6	(0.2-12.4)	0.9	(0.3-2.3)
Proteinuria, gram per day	0 to 0.3	1.0	(reference)	1.0	(reference)
	>0.3 to 3.5	0.3	(0.1-1.1)	1.3	(0.7-2.3)
	≥3.5	0.8	(0.2-3.2)	1.2	(0.6-2.6)
Albumin, g/L	<30.1	1.0	(reference)	1.0	(reference)
	≥30.1 to 35.5	0.8	(0.2-2.5)	0.7	(0.5-1.2)
	>35.5	0.4	(0.1-1.6)	0.8	(0.5-1.2)
Cholesterol, mmol/L	<3.9	1.0	(reference)	1.0	(reference)
	≥3.9 to 5.0	0.9	(0.2-3.6)	1.1	(0.6-1.9)
	>5.0	1.6	(0.5-5.7)	1.6	(1.0-2.8)
Triglycerides, mmol/L	<1.4	1.0	(reference)	1.0	(0.6-1.7)
	≥1.4 to 2.3	1.1	(0.2-5.0)	0.8	(0.5-1.4)
	>2.3	1.3	(0.3-5.7)	1.1	(0.7-1.9)

NE indicates not estimable. *hazard ratios adjusted for age and sex.

Table 3. History of venous and arterial thrombosis prior to start of dialysis treatment and mortality risk after adjustment for age and sex

	All-cause mortality Hazard ratios* (95% CI)		CV mortality Hazard ratios* (95% CI)		Non CV mortality Hazard ratios* (95% CI)	
No history venous or arterial thrombosis	1.0	(reference)	1.0	(reference)	1.0	(reference)
History of venous thrombosis	1.9	(1.1-3.3)	2.0	(0.9-4.4)	1.8	(0.8-4.0)
History of arterial thrombosis	1.9	(1.4-2.6)	2.4	(1.6-3.7)	1.5	(1.0-2.4)

*hazard ratios adjusted for age, sex, diabetes, and primary kidney disease.

DISCUSSION

In the present study, we observed that dialysis patients had absolute risks of more than one percent per year for venous thrombosis, myocardial infarction and ischemic stroke, with 6-fold increase of venous thrombosis, 8-fold increase of ischemic stroke, and 12-fold increase of myocardial infarction risk as compared to the age- and sex-weighted incidence rates in the general population. Finally, our data showed a strong association between a history of venous and arterial thrombosis prior to the start of dialysis and mortality during dialysis.

To our knowledge, this the first study that assessed the incidence of both deep vein thrombosis and pulmonary embolism in end-stage renal disease patients. One other study has examined the incidence of only pulmonary embolisms in end-stage renal disease patients. It showed that dialysis patients had a 2.3-fold increased risk for pulmonary embolism,[21] which is lower than in our study. However, as they only assessed pulmonary embolism in case of primary discharge diagnosis in the first year of dialysis, this could have resulted in an underestimation of the number of pulmonary embolisms. The observed risk of venous thrombosis in dialysis patients in our cohort is in contrast with previous autopsy studies.[27-30] These studies showed that pulmonary embolism was less common in dialysis patients than in non-dialysis patients.[27-30] However, the incidence of venous thrombosis may be underestimated in these autopsy studies, since only a small and selective proportion of dialysis patients undergo postmortem examination. Furthermore, postmortem diagnosis often provides little information about the clinical significance of thrombotic events. The increased risk for myocardial infarction and ischemic stroke in our Dutch cohort of dialysis patients is in line with previous studies.[14-20] Studies revealed that cardiovascular mortality rates were 8 to 20 times higher than in the general population.[15-17]

A possible explanation for the increased risk of venous thrombosis is the high rate of hospitalization, surgery, and immobilization resulting in stasis of the blood and in subsequent venous thrombosis. However, we also found an increased incidence of unprovoked venous

thrombosis suggesting that also other factors play a role in the development of venous thrombosis in dialysis patients. One of these other factors could be hypercoagulability. Several studies have shown that there is a hypercoagulable state in dialysis patients.[31,32] Another explanation for the increased risk of venous thrombosis in dialysis patients could be that the high usually rate of thrombus formation in grafts and fistulas in hemodialysis patients may cause pulmonary embolisms through dislodgement of thrombi.[33] An important finding that strengthens this hypothesis was that venous thrombosis was more frequent in hemodialysis patients than in peritoneal dialysis patients. Moreover, deep vein thrombosis and pulmonary embolism occurred in a similar frequency in this cohort of dialysis patients, whereas in the general population deep vein thrombosis is twice as frequent as pulmonary embolism.[23] In addition, one patient had a symptomatic pulmonary embolism shortly after a thrombectomy of a thrombosed dialysis shunt.

Recent studies have challenged the historical dichotomy of arterial and venous thrombosis as two different entities with distinct risk factors.[2-6] Indeed, arterial cardiovascular risk factors such as hypertension, smoking, and diabetes appeared to be risk factors for venous thrombosis as well.[7-11] In our study, venous and arterial thrombosis did not share risk factors in these dialysis patients, except for a history of venous thrombosis prior to the start of dialysis which was associated with both venous and arterial thrombosis. “Classic” cardiovascular risk factors in the general population, such as an increased age, diabetic nephropathy, renal vascular disease, history of arterial thrombosis, diabetes as comorbidity, and highest tertiles of cholesterol were associated with subsequent arterial thrombosis and not with venous thrombosis. Malignancy, a “classic” risk factor for venous thrombosis in the general population was associated with a non-significantly increased risk of subsequent venous thrombosis. Furthermore, we found a non-significant inverse association between serum albumin levels and venous thrombosis. Also in patients with nephrotic syndrome, serum albumin has been inversely associated with venous thrombosis.[34,35]

We showed that both a history of arterial thrombosis and venous thrombosis before the start of dialysis increased the mortality risk during dialysis. Prior studies also found that dialysis patients who had suffered cardiovascular disease had a poor long-term survival.[36,37] This finding is in agreement with previous studies that showed that venous thrombosis was associated with an increased risk for arterial thrombosis[3-5] and an increased long-term mortality risk in the general population.[38] Therefore, it is tempting to suggest that a history of venous thrombosis before the start of dialysis could be marker of underlying atherosclerosis which results in an increased risk of subsequent arterial thrombosis and an increased mortality risk. Atherosclerosis in patients with a history of venous or arterial thrombosis could also explain why the hazard ratios were higher for cardiovascular mortality than for non-cardiovascular mortality. We did not find an

association between a history of arterial thrombosis and subsequent venous thrombosis. This might be explained by the high prevalence of anticoagulation use in patients with a history of arterial thrombosis preventing also venous thrombosis. Indeed, after exclusion of vitamin K antagonist users, the risk of venous thrombosis for patients with a history of arterial thrombosis was 1.6-fold increased, but as numbers in this subgroup analysis became small, the results should be handled with caution.

A strength of this study is its prospective design in which objectively confirmed venous and arterial thrombotic events were considered as outcome measures. Nevertheless, our study has some potential limitations that should be addressed. A limitation of this study was that we could not measure levels or activity of coagulation factors or markers of hypercoagulability to investigate the role of these factors in the development of thrombotic events in dialysis patients. Another limitation of this study was that confidence intervals around the hazard ratios were wide for risk factors of venous and arterial thrombosis, indicating a limited power for detecting underlying risk factors for venous and arterial thrombosis in dialysis patients. Small numbers also restricted us to not perform further analyses of potential risk factors (such as hemodialysis or peritoneal dialysis) on mortality in patients with previous venous or arterial thrombosis. Nevertheless, this study is the largest to date that analyzed risk factors for both venous and arterial thrombosis and subsequent mortality in dialysis patients.

In conclusion, we showed that dialysis patients had high risks for venous and arterial thrombosis, while occurrence of these thrombotic diseases prior to the start of dialysis was associated with an increased mortality risk in this patient group. Furthermore, we showed that venous and arterial thrombosis did not share risk factors in these dialysis patients.

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Chapter 7

Mortality due to pulmonary embolism, myocardial infarction, and stroke among incident dialysis patients

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ABSTRACT

Background: It has been suggested that dialysis patients have lower mortality rates for pulmonary embolism than the general population, because of platelet dysfunction and bleeding tendency. However, there is limited information whether dialysis is indeed associated with a decreased mortality risk from pulmonary embolism. The aim of our study was to evaluate whether mortality rate ratios for pulmonary embolism were lower than for myocardial infarction and stroke in dialysis patients compared with the general population.

Methods: Cardiovascular causes of death for 130 439 incident dialysis patients registered in the ERA-EDTA Registry were compared with the cardiovascular causes of death for the European general population.

Results: The age- and sex-standardized mortality rate (SMR) from pulmonary embolism was 12.2 (95%CI 10.2-14.6) times higher in dialysis patients than in the general population. The SMRs in dialysis patients compared with the general population were 11.0 (95% CI 10.6-11.4) for myocardial infarction, 8.4 (95% CI 8.0-8.8) for stroke, and 8.3 (95% CI 8.0-8.5) for other cardiovascular diseases. In dialysis patients, primary kidney disease due to diabetes was associated with an increased mortality risk due to pulmonary embolism (HR 1.9; 95% CI 1.0-3.8), myocardial infarction (HR 4.1; 95% CI 3.4-4.9), stroke (HR 3.5; 95% CI 2.8-4.4), and other cardiovascular causes of death (HR 3.4; 95% CI 2.9-3.9) compared with patients with polycystic kidney disease.

Conclusion: Dialysis patients were found to have an unexpected highly increased mortality rate for pulmonary embolism and increased mortality rates for myocardial infarction and stroke.

INTRODUCTION

End-stage renal disease patients who receive dialysis treatment have a markedly increased risk of death with a cardiovascular mortality risk that is 8-20 times higher than in the general population.[1-6] However, there is limited information on the contribution of various specific causes of cardiovascular death such as pulmonary embolism, myocardial infarction and stroke to the excess risk. It has been suggested that dialysis patients have a lower risk for pulmonary embolism than the general population, because of platelet dysfunction and bleeding tendency.[7,8] In support of this notion, autopsy studies have shown pulmonary embolism to be less common in dialysis patients than in non-dialysis patients.[9-12] However, epidemiological studies investigating the risk of mortality due to pulmonary embolism in dialysis patients are lacking. While studies in dialysis patients have shown an increased risk for myocardial infarction[13] and stroke,[14,15] the specific mortality risks due to these causes have not been adequately dissected.

Based on the previous autopsy studies on pulmonary embolism, we hypothesized that mortality rate ratios for pulmonary embolism would be lower than those for myocardial infarction and stroke in dialysis patients compared with the general population. Therefore, our aim was to assess the rates of mortality from myocardial infarction, stroke, and pulmonary embolism in a large cohort of incident dialysis patients and compare them with those in the general population. Finally, we set out to investigate the risk factors for death from these specific cardiovascular causes in the dialysis population.

METHODS

Dialysis patients

The study cohort consisted of incident dialysis patients derived from the European Renal Association-European Dialysis and Transplant Association (ERA-EDTA) Registry.[16] This cohort included patients from national and regional registries in 11 European countries: Austria, Dutch- and French-speaking Belgium, Denmark, Finland, Greece, Iceland, Calabria (Italy), the Netherlands, Norway and the autonomous communities of Andalusia, Asturias, Basque country, Catalonia, Castile-La Mancha, Castile and Leon, Extremadura, and Valencian region in Spain, and Sweden. The ERA-EDTA Registry collects data on renal replacement therapy, including date of birth, sex, primary kidney disease, date of start of renal replacement therapy, dialysis modality at baseline and during follow-up, and date and cause of death. Primary kidney disease was classified according to the coding system of the ERA-EDTA.[17] We grouped patients into nine classes of primary kidney disease: polycystic kidney disease, glomerulonephritis, pyelonephritis, hypertension, renal vascular disease, diabetes, multi-system disease (renal

vascular disease due to polyarteritis, granulomatous polyangiitis, glomerulonephritis related to liver cirrhosis, cryoglobulinaemic glomerulonephritis, myelomatosis, amyloidosis, systemic lupus erythematosus, Henoch-Schoenlein purpura, Goodpasture's syndrome, and systemic sclerosis), miscellaneous, and missing/unknown. Patients were included if they originated from registries reporting less than 25% missing or unknown causes of death. We included patients who initiated dialysis between January 1, 1994, and December 31, 2005, and followed them for a maximum of 3 years from onset of dialysis until December 31, 2008, or until death or censoring (i.e. recovery of renal function, kidney transplantation or loss to follow-up).

Mortality in dialysis patients

We classified the causes of death according to the coding system of the ERA-EDTA, which is a standardized classification of causes of death in dialysis patients.[17] Cardiovascular mortality was defined as a death attributable to pulmonary embolism (ERA-EDTA code 21), myocardial infarction (ERA-EDTA code 11), stroke (ERA-EDTA code 22), and other cardiovascular causes [cardiac arrest / sudden death (ERA-EDTA code 15), fluid overload / pulmonary edema (ERA-EDTA codes 18), hypertensive cardiac failure (ERA-EDTA codes 16), other causes of cardiac failure (ERA-EDTA codes 14)]. Unknown (ERA-EDTA code 0) and missing causes of death were defined as unknown. All other causes of death were defined as non-cardiovascular.

Mortality in the general population

Mortality data obtained from the general population in the corresponding 11 countries (or regions) that contributed data on dialysis patients were used as reference. These mortality data, derived from the national cause of death statistics, were obtained from the World Health Organization (WHO). The WHO provides mortality data coded according to the International Statistical Classification of Diseases (ICD), stratified by age categories, sex, and calendar year.

Cardiovascular mortality in the general population was defined as death from diseases of the circulatory system (ICD-9 codes 390-459; ICD-10 codes I00-I99),[3] i.e. pulmonary embolism (ICD-9 codes 415.1; ICD-10 codes I26),[18] myocardial infarction (ICD-9 code 410; ICD-10 codes I21-I22),[19] stroke (ICD-9 codes 430-434, 436; ICD-10 codes I60-I64)[20], and other causes of cardiovascular death (ICD-9 codes 390-459, except 415.1, 410, 430-434, and 436; ICD-10 codes I00-I99, except I26, I21-I22, and I60-I64). Ill-defined and unknown causes of mortality (ICD-9 codes 797-799; ICD-10 codes R96-R99) were regarded as unknown cause of death in the general population, while all other codes (all ICD-9 codes except 390-459 and 797-799 and all ICD-10 codes except I00-I99 and R96-R99) were regarded as non-cardiovascular causes of death.

Statistical analysis

Continuous variables are presented as means with standard deviation (SD). Data were stratified by age-categories (20-24 years, 25-34 years, 35-44 years, 45-54 years, 55-64 years, 65-74 years, 75-84, and ≥85 years) and sex.

The rates of mortality from all causes and specific cardiovascular causes (pulmonary embolism, myocardial infarction, stroke, and other) in each age category were calculated by dividing the number of patients who died due to cardiovascular causes by the total observation time at risk in each age stratum, for both the dialysis patients and the general population. The time at risk in the general population was calculated using the demographic large-scale method.[3] Using this method, person-time at risk in the general population of the 11 countries from which dialysis patients were included, was calculated as the sum of the mean size of the general population in the subsequent calendar years.

Furthermore, we calculated crude mortality rate ratios with 95% CIs by dividing the mortality rates in dialysis patients by the mortality rates in the general population. In addition, age- and sex-standardized mortality rate ratios were calculated using direct standardization with the general population as reference. We also did a sensitivity analysis in which age- and sex-standardized mortality rate ratios with 95% CIs were calculated for pulmonary embolism, myocardial infarction, and stroke after changing all unknown codes of death in the general population into pulmonary embolism, myocardial infarction, or stroke, respectively. Finally, among incident dialysis patients, we calculated hazard ratios (HRs) with 95% CIs to evaluate the effect of age, sex, and primary kidney disease at baseline on death due to pulmonary embolism, myocardial infarction, stroke, and other cardiovascular diseases using Cox regression. We also used Cox regression to evaluate the effect of dialysis modality (hemodialysis or peritoneal dialysis) at 3 months after the beginning of renal replacement therapy on death due to pulmonary embolism, myocardial infarction, stroke, and other cardiovascular diseases. SPSS statistical software (version 18.0; SPSS, Chicago, Illinois) was used for the analysis.

RESULTS

In this study, we included 130 439 dialysis patients from 11 countries who began dialysis between January 1, 1994, and December 31, 2005. The mean age of the dialysis patients was 63.1 years, 61.0% were male, in 22.3% diabetes was the cause of kidney disease, and 84.1% started hemodialysis as the initial dialysis modality (Table 1). The mean follow-up of dialysis patients was 2.0 years resulting in a total observation time of 260 772 years. During the observation period, 23.7% of the patients underwent renal transplantation.

Table 1. Baseline characteristics of dialysis patients

Characteristic	Age category at onset of dialysis, n (%)								
	All (n=130,439)	**20-24 y (n=1,686)**	**25-34 y (n= 5,738)**	**35-44 y (n=9,932)**	**45-54 y (n=17,189)**	**55-64 y (n=26,405)**	**65-74 y (n=40,030)**	**75-84 y (n=26,737)**	**≥85 y (n=2,722)**
Age, mean (SD), y	63.1 (14.7)	22.7 (1.4)	30.5 (2.8)	40.4 (2.9)	50.4 (2.8)	60.4 (2.9)	70.2 (2.8)	78.9 (2.6)	87.5 (2.3)
Sex (%)									
Female	39.0	35.3	39.3	36.1	36.8	37.1	39.7	41.7	44.0
Male	61.0	64.7	60.7	63.9	63.2	62.9	60.3	58.3	56.0
Primary kidney disease (%)									
Polycystic kidney disease	5.9	0.5	2.1	9.8	13.4	7.9	3.9	2.4	1.3
Pyelonephritis	7.2	16.8	11.4	7.3	6.4	6.3	7.0	7.4	7.5
Glomerulonephritis	13.5	31.9	29.5	24.7	19.0	13.5	9.7	7.7	5.3
Hypertension	11.0	3.1	5.2	6.8	7.8	9.4	11.9	16.0	18.5
Renal vascular disease	6.8	0.7	0.8	1.2	2.7	4.8	8.3	11.9	15.2
Diabetes	22.3	3.0	17.3	21.0	22.4	27.0	25.4	17.1	8.7
Miscellaneous	8.5	17.4	10.4	8.8	8.5	9.3	8.2	7.2	6.6
Multisystem disease	7.0	12.2	8.8	6.9	6.6	6.9	7.2	6.6	4.6
Unknown	17.8	14.5	14.6	13.4	13.1	14.9	18.3	23.8	32.2
Dialysis modality (%)									
Peritoneal dialysis	15.9	20.6	24.6	25.1	22.1	17.7	12.9	9.8	7.6
Hemodialysis	84.1	79.4	75.4	74.9	77.9	82.3	87.1	90.2	92.4
Renal transplantation* (%)	23.7	80.7	75.1	65.2	50.1	28.4	6.3	0.4	0.1
Follow-up, mean (SD), y	2.0 (1.1)	1.9 (1.1)	1.9 (1.0)	2.0 (1.0)	2.1 (1.0)	2.1 (1.0)	2.0 (1.1)	1.8 (1.1)	1.4 (1.1)

*transplantation during follow-up

The general population yielded an observation time of 1140.2 million person-years. The mean age of the general population was lower than the mean age of the dialysis patients. There were fewer men in the general population than in the dialysis patients.

During follow-up, 50 765 of the 130 439 dialysis patients died (Table 2). Among the deceased patients, cardiovascular diseases were the cause of death in 39.8% of cases: pulmonary embolism 0.7%, myocardial infarction 11.4%, stroke 7.3%, and other cardiovascular cause of death 20.3% (including cardiac arrest/ sudden death 12.3% and other than cardiac arrest/ sudden death 8.0%). Death from non-cardiovascular causes occurred in 46.5% of the patients, while the cause of death was unknown in 13.8%. There was a similar pattern of cardiovascular causes of death (pulmonary embolism, myocardial infarction, stroke, and other cardiovascular cause of death) across the age groups. In the general population, 13 739 478 persons died during the study period. Of those, 40.1% died from cardiovascular diseases (pulmonary embolism 0.5%, myocardial infarction 8.6%, stroke 9.0%, and other cardiovascular cause of death 21.9%), 57.9% from non-cardiovascular diseases, and 2.0% from unknown causes.

Both in the general population and the dialysis population, the mortality rates for pulmonary embolism, myocardial infarction, stroke, and other cardiovascular causes of death increased with age (Figure 1).

Table 3 shows the mortality rates for total and cause specific (pulmonary embolism, myocardial infarction, stroke, and other) cardiovascular mortality in different age categories stratified by sex. In both the dialysis patients and the general population, mortality rates due to myocardial infarction and due to other cardiovascular causes of death were higher in men than in women, while women were at higher risk of death due to stroke and pulmonary embolism than men. The total cardiovascular mortality rate was 77.4 per 1000 person-years in dialysis patients and 4.8 per 1000 person-years in the general population. The age- and sex-standardized cardiovascular mortality rate was 8.9 (95% CI 8.7-9.1) times higher in dialysis patients than in the general population.

Table 2. Causes of death in the first three years of dialysis treatment

Dialysis patients	All (n=130,439)		Age category at death, N (%)															
			20-24 y (n=1,008)		25-34 y (n=5,039)		35-44 y (n=8,845)		45-54 y (n=15,415)		55-64 y (n=24,075)		65-74 y (n=38,064)		75-84 y (n=33,080)		≥85 y (n=4,913)	
Total deaths, n (%)	**50,765**	**(100)**	**57**	**(100)**	**346**	**(100)**	**1,009**	**(100)**	**2,918**	**(100)**	**7,371**	**(100)**	**17,759**	**(100)**	**18,359**	**(100)**	**2,946**	**(100)**
CV*, n (%)	**20,187**	**(39.8)**	**11**	**(19.3)**	**110**	**(31.8)**	**411**	**(40.7)**	**1,185**	**(40.6)**	**2,981**	**(40.4)**	**7,231**	**(40.7)**	**7,185**	**(39.1)**	**1,073**	**(36.4)**
Pulmonary embolism	365	(0.7)	1	(1.8)	1	(0.3)	8	(0.8)	18	(0.6)	51	(0.7)	136	(0.8)	137	(0.8)	13	(0.4)
Myocardial infarction	5,812	(11.4)	0	(0.0)	16	(4.6)	97	(9.6)	341	(11.7)	960	(13.0)	2,165	(12.2)	2,032	(11.1)	201	(6.8)
Stroke	3,699	(7.3)	2	(3.5)	41	(11.9)	107	(10.6)	253	(8.7)	520	(7.4)	1,254	(7.1)	1,288	(7.0)	234	(7.9)
Other	10,311	(20.3)	8	(14.0)	52	(15.0)	199	(19.7)	573	(19.6)	1,450	(19.7)	3,676	(20.7)	3,728	(20.3)	625	(21.2)
Cardiac arrest/ sudden death	6,235	(12.3)	5	(8.8)	37	(10.7)	144	(14.3)	382	(13.1)	903	(12.3)	2,200	(12.4)	2,163	(11.8)	401	(13.6)
Other than cardiac arrest/ sudden death	4,076	(8.0)	3	(5.3)	15	(4.3)	55	(5.5)	191	(6.5)	547	(7.4)	1,476	(8.3)	1,565	(8.5)	224	(7.6)
Non-CV*, n (%)	**23,597**	**(46.5)**	**35**	**(61.4)**	**185**	**(53.5)**	**447**	**(44.3)**	**1,321**	**(45.3)**	**3,397**	**(46.1)**	**8,113**	**(45.7)**	**8,639**	**(47.1)**	**1,460**	**(49.6)**
Unknown, n (%)	**6,981**	**(13.8)**	**11**	**(19.3)**	**51**	**(14.7)**	**151**	**(15.0)**	**412**	**(14.1)**	**993**	**(13.5)**	**2,415**	**(13.6)**	**2,535**	**(13.8)**	**413**	**(14.0)**

*CV, cardiovascular disease

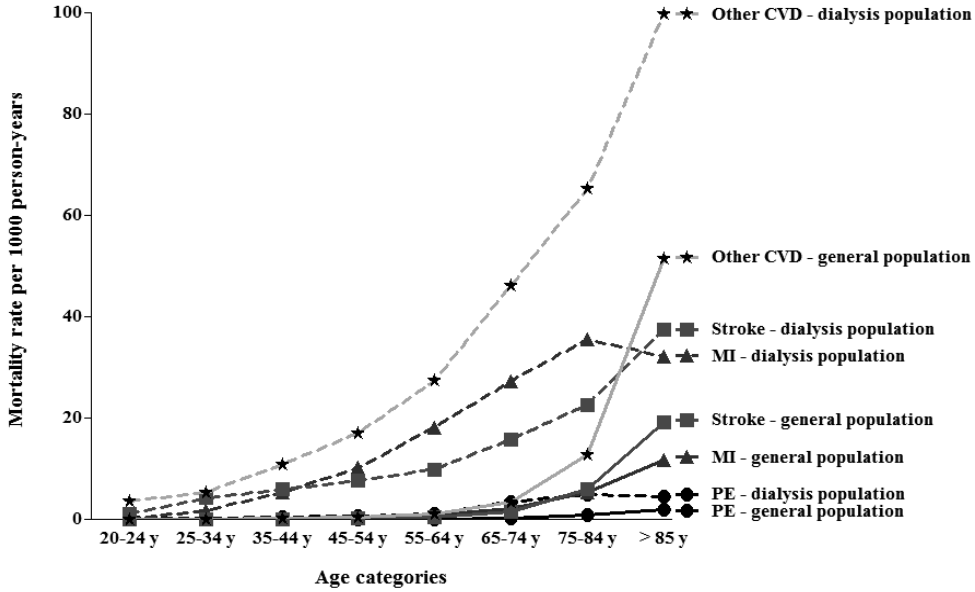

Figure 1. Mortality rates due to pulmonary embolism, myocardial infarction, stroke, and other cardiovascular diseases in dialysis patients and in the general population for different age categories

PE, pulmonary embolism; MI, myocardial infarction; CVD, cardiovascular disease; y, years

Compared with the general population, the age- and sex-standardized mortality rate ratios in dialysis patients were 12.2 (95% CI 10.2-14.6) for pulmonary embolism (1.4 among dialysis patients versus 0.1 among the general population per 1000 person-years, respectively), 11.0 (95% CI 10.6-11.4) for myocardial infarction (22.3 versus 1.0 per 1000 person-years, respectively), 8.4 (95% CI 8.0-8.8) for stroke (14.2 versus 1.1 per 1000 person-years, respectively), and 8.3 (95% CI 8.0-8.5) for other cardiovascular causes of death (39.5 versus 2.6 per 1000 person-years, respectively). The mortality rates for all cardiovascular causes of death were highest in the first 6 months after initiation of dialysis: mortality rate 1.9 per 1000 person-years for pulmonary embolism, 26.2 per 1000 person-years for myocardial infarction, 15.5 per 1000 person-years for stroke, and 51.6 per 1000 person-years for other cardiovascular causes of death.

In a worst case scenario, in which we changed all unknown codes of death in the general population into pulmonary embolism, myocardial infarction, or stroke, age- and sex-standardized mortality rate ratios were still increased for respectively pulmonary embolism (2.7; 95% CI 2.3-3.3), for myocardial infarction (8.4; 95% CI 8.0-8.8) and stroke (6.8; 95% CI 6.5-7.2).

Table 3. Total and cause-specific mortality rates per 1000 person-years in dialysis patients compared with the general population

		Age category, N (%)								
Characteristic		**All**	**20-24 y**	**25-34 y**	**35-44 y**	**45-54 y**	**55-64 y**	**65-74 y**	**75-84 y**	**≥85 y**
Total cardiovascular mortality										
Dialysis patients	Male	80.5	5.5	9.9	22.9	38.2	60.6	95.6	131.8	176.6
	Female	72.6	3.6	12.9	20.9	30.1	48.7	83.5	116.9	164.1
	Total	77.4	4.8	11.1	22.1	35.2	56.2	90.7	125.6	171.1
General	Male	4.6	0.0	0.1	0.3	1.1	3.2	9.7	28.9	86.0
Population	Female	5.0	0.0	0.0	0.1	0.4	1.1	4.7	21.0	82.0
	Total	4.8	0.0	0.1	0.2	0.7	2.1	7.0	24.1	83.2
Unstandardized mortality rate ratio 16.0 (95% CI 15.8-16.3)					**Age- and sex-standardized mortality rate ratio 8.9 (95% CI 8.7-9.1)**					
Pulmonary embolism										
Dialysis patients	Male	1.1	0.0	0.2	0.2	0.6	0.9	1.4	2.0	1.7
	Female	1.8	1.2	0.0	0.9	0.5	1.1	2.2	3.0	2.6
	Total	1.4	0.4	0.1	0.4	0.5	1.0	1.7	2.4	2.1
General	Male	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.9
Population	Female	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.4	0.9
	Total	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.4	0.9
Unstandardized mortality rate ratio 20.2 (95% CI 18.3-22.4)					**Age- and sex-standardized mortality rate ratio 12.2 (95% CI 10.2-14.6)**					
Myocardial infarction										
Dialysis patients	Male	25.2	0.0	1.3	5.3	11.8	21.1	30.6	41.0	37.4
	Female	17.8	0.0	2.0	5.1	7.2	13.0	22.1	27.9	25.2
	Total	22.3	0.0	1.6	5.2	10.1	18.1	27.2	35.5	32.1
General	Male	1.2	0.0	0.0	0.1	0.5	1.2	3.1	7.1	14.6
Population	Female	0.9	0.0	0.0	0.0	0.1	0.3	1.2	4.0	10.5
	Total	1.0	0.0	0.0	0.1	0.3	0.7	2.0	5.2	11.7
Unstandardized mortality rate ratio 21.5 (95% CI 21.0-22.1)					**Age- and sex-standardized mortality rate ratio 11.0 (95% CI 10.6-11.4)**					
Stroke										
Dialysis patients	Male	13.2	1.4	3.7	5.6	8.3	9.3	14.7	20.8	33.4
	Female	15.7	0.0	4.8	6.0	6.1	10.6	17.2	25.0	42.3
	Total	14.2	0.9	4.1	5.8	7.5	9.8	15.7	22.5	37.3
General	Male	0.9	0.0	0.0	0.1	0.2	0.5	1.7	6.2	18.4
Population	Female	1.3	0.0	0.0	0.0	0.1	0.3	1.2	5.6	19.3
	Total	1.1	0.0	0.0	0.0	0.1	0.4	1.4	5.8	19.0
Unstandardized mortality rate ratio 13.1 (95% CI 12.7-13.5)					**Age- and sex-standardized mortality rate ratio 8.4 (95% CI 8.0-8.8)**					
Other Cardiovascular disease										
Dialysis patients	Male	41.0	4.1	4.7	11.7	17.4	29.3	48.9	68.0	104.0
	Female	37.3	2.4	6.1	9.0	16.3	23.9	42.0	61.1	94.1
	Total	39.5	3.5	5.2	10.7	17.0	27.3	46.1	65.2	99.7
General	Male	2.4	0.0	0.0	0.1	0.5	1.4	4.7	15.2	52.2
Population	Female	2.9	0.0	0.0	0.1	0.2	0.5	2.3	11.0	51.2
	Total	2.6	0.0	0.0	0.1	0.3	1.0	3.4	12.7	51.5
Unstandardized mortality rate ratio 15.0 (95% CI 14.7-15.3)					**Age- and sex-standardized mortality rate ratio 8.3 (95% CI 8.0-8.5)**					

In dialysis patients, older age at the beginning of dialysis (≥85 years) was associated with an increased risk of pulmonary embolism (HR 7.8; 95% CI 3.3-18.6), myocardial infarction (HR 9.6; 95% CI 7.5-12.3), stroke (HR 8.9; 95% CI 7.2-11.2), and other cardiovascular causes of death (HR 12.3; 95% 10.6-14.3) when compared with patients who were younger at onset of dialysis (20-44 years) (Table 4). Male sex was associated with an increased risk of myocardial infarction (HR 1.4; 95% CI 1.3-1.5) and other cardiovascular causes of death (HR 1.1; 95% 1.1-1.2), whereas males had a decreased risk of death due to pulmonary embolism (HR 0.6; 95% CI 0.5-0.8) and stroke (HR 0.9; 95% CI 0.8-0.9). Both diabetes and multi-system diseases were associated with an increased risk of pulmonary embolism (HR 1.9; 95% CI 1.0-3.8 and HR 3.2; 95% CI 1.6-6.4, respectively), myocardial infarction (HR 4.1; 95% CI 3.4-4.9 and HR 2.2; 95% CI 1.7-2.7, respectively), stroke (HR 3.5; 95% CI 2.8-4.4 and HR 2.8; 95% CI 2.1-3.6, respectively), and other cardiovascular causes of death (HR 3.4; 95% CI 2.9-3.9 and HR 3.4; 95% CI 2.9-4.0, respectively), as compared with polycystic kidney disease. Dialysis modality was not associated with specific cardiovascular causes of death. Risk factors were the same for different causes of death in the other cardiovascular causes of death group.

Table 4. Risk factors for mortality due to specific cardiovascular diseases in dialysis patients

	Hazard ratio (95% CI) Pulmonary embolism		Hazard ratio (95% CI) Myocardial infarction		Hazard ratio (95% CI) Stroke		Hazard ratio (95% CI) Other CV	
Age categories at onset of dialysis*								
20-44	1	(reference)	1	(reference)	1	(reference)	1	(reference)
45-54	1.6	(0.8-3.4)	2.6	(2.1-3.1)	1.5	(1.2-1.8)	1.9	(1.7-2.2)
55-64	3.1	(1.6-5.8)	4.6	(3.8-5.5)	2.2	(1.8-2.6)	3.2	(2.8-3.6)
65-74	5.1	(2.8-9.4)	7.4	(6.2-8.8)	3.4	(2.9-4.0)	5.3	(4.7-6.0)
75-84	8.0	(4.3-14.8)	9.4	(7.9-11.3)	5.0	(4.2-5.9)	7.7	(6.9-8.7)
≥85	7.8	(3.3-18.6)	9.6	(7.5-12.3)	8.9	(7.2-11.2)	12.3	(10.6-14.3)
Sex								
Female	1	(reference)	1	(reference)	1	(reference)	1	(reference)
Male	0.6	(0.5-0.8)	1.4	(1.3-1.5)	0.9	(0.8-0.9)	1.1	(1.1-1.2)
Primary kidney disease†								
Polycystic kidney disease	1	(reference)	1	(reference)	1	(reference)	1	(reference)
Glomerulonephritis	1.3	(0.7-2.7)	1.3	(1.1-1.7)	1.6	(1.3-2.1)	1.5	(1.3-1.8)
Pyelonephritis	1.7	(0.8-3.5)	1.3	(1.1-1.7)	1.6	(1.2-2.1)	1.6	(1.3-1.9)
Hypertension	1.2	(0.6-2.4)	2.5	(2.1-3.1)	2.3	(1.8-2.9)	2.3	(2.0-2.7)
Renal vascular disease	1.8	(0.9-3.8)	2.9	(2.3-3.5)	2.7	(2.1-3.4)	3.1	(2.7-3.7)
Diabetes	1.9	(1.0-3.8)	4.1	(3.4-4.9)	3.5	(2.8-4.4)	3.4	(2.9-3.9)
Miscellaneous	1.9	(0.9-3.8)	1.6	(1.3-1.9)	2.1	(1.6-2.7)	2.0	(1.7-2.3)
Multisystem disease	3.2	(1.6-6.4)	2.2	(1.7-2.7)	2.8	(2.1-3.6)	3.4	(2.9-4.0)
Unknown	1.6	(0.8-3.1)	1.9	(1.6-2.4)	2.2	(1.8-2.8)	2.3	(2.0-2.7)
Dialysis modality‡								
Peritoneal dialysis	1	(reference)	1	(reference)	1	(reference)	1	(reference)
Hemodialysis	0.8	(0.6-1.1)	1.0	(0.9-1.1)	1.0	(0.9-1.1)	1.0	(0.9-1.1)

CV, cardiovascular disease; *Adjusted for sex, calendar year, and country; †Adjusted for age, sex, calendar year, and country; ‡Adjusted for age, sex, primary kidney disease, calendar year, and country

DISCUSSION

We found an unexpected highly increased risk of pulmonary embolism in dialysis patients as compared with the general population and an elevated risk of myocardial infarction and stroke in dialysis patients as compared with the general population. The total cardiovascular mortality rate in the 130 439 dialysis patients was 77.4 per 1000 person-years of which 1.4 per 1000 person-years could be attributed to pulmonary embolism, 22.3 per 1000 person-years to myocardial infarction, 14.2 per 1000 person-years to stroke, and 39.5 per 1000 person-years to other cardiovascular causes. The age- and sex-standardized mortality rates in dialysis patients were 12.2-fold increased for pulmonary embolism, 11.0-fold increased for myocardial infarction, 8.9-fold increased for stroke, and 8.3-fold increased for other cardiovascular causes of death compared with the general population.

The previous studies have investigated the total cardiovascular mortality risk in dialysis patients compared with the general population,[1,3,6] but there is limited information on the contribution of the various specific causes of cardiovascular death to the excess risk. The age- and sex-standardized mortality rates in dialysis patients as compared with the general population were comparable to a previous European study.[21] While two previous studies suggested an increased incidence of pulmonary embolism in the first year of dialysis,[22,23] there is limited information on the mortality associated with pulmonary embolism and whether it contributes to the increased cardiovascular mortality risk in dialysis patients. Our results contradict the findings of autopsy studies on pulmonary embolism.[9-12] These studies showed that pulmonary embolism was less common in dialysis patients (prevalences ranging from 0% to 6.5%) than in non-dialysis patients (prevalences ranging from 8.2% to 16.0%). In our study, 0.7% of deaths in dialysis patients were caused by pulmonary embolism compared with 0.5% in the general population. Comparison of our findings with those from the aforementioned autopsy series is hampered for several reasons. First, the use of autopsy series to investigate pulmonary embolism could lead to selection bias caused by different indications for autopsy in dialysis patients and non-dialysis patients. Moreover, postmortem diagnosis often provides little information about the clinical significance of pulmonary embolism and information on whether it contributed to death is often lacking.

Our finding that the mortality rate ratio was highest for pulmonary embolism was a surprise. Although pulmonary embolism as a cause of death is less common, clinicians should be aware of the increased risk of this disorder in dialysis patients, since it is the most common preventable cause of hospital death.[24] In contrast to myocardial infarction and stroke, pulmonary embolism can be prevented by administering prophylactic anticoagulation therapy in patients considered to be at increased risk.[25] Especially high-risk groups, including elderly dialysis patients with diabetes or multi-system disease as shown in our study, could benefit from thromboprophylaxis. Nevertheless, further studies are needed to show whether thromboprophylaxis is cost-effective and safe in high-risk dialysis patients, given the increased bleeding risk associated with anticoagulation and given its potential role in the generation of vascular calcification. The increased mortality rate for myocardial infarction,[2,13,26,27] stroke,[14,15,27,28] and other cardiovascular diseases[2,4,5] in our cohort of dialysis patients is in line with previous studies that showed an increased incidence of myocardial infarction, stroke, and other cardiovascular events.

Our results show that death due to pulmonary embolism was associated with increased age, female sex, and diabetes. Female sex and increased age are also important risk factors for pulmonary embolism in the general population.[29] Studies on the association between diabetes and pulmonary embolism in the general population have shown conflicting results,[30,31] but our

data show that diabetes is a risk factor in dialysis patients. We did not find an association between treatment modality (hemodialysis or peritoneal dialysis) and mortality due to pulmonary embolism. It could be suggested that the mortality risk of pulmonary embolism should be theoretically lower for hemodialysis patients than for peritoneal dialysis patients, because of anticoagulation use during hemodialysis sessions to prevent clot formation. However, peritoneal dialysis patients could have less comorbidities than hemodialysis patients at baseline explaining the similar mortality rates for hemodialysis and peritoneal dialysis patients. We had no information about comorbidities to investigate this. Our findings that older age, male sex, and diabetes as cause of end-stage renal disease were associated with an increased risk of myocardial infarction in dialysis patients is consistent with previous studies.[1,13] The association between stroke and increased age, female sex, and diabetes as cause of end-stage renal disease in our study was also in agreement with previous studies in dialysis patients.[14,15,27,32]

The unexpected increased mortality risk of pulmonary embolism could reflect an increased incidence of pulmonary embolism in dialysis patients.[22,23] Furthermore, an increased fatality rate of pulmonary embolism in dialysis patients could lead to an increased mortality risk due to pulmonary embolism.[33] A previous study showed that patients with a severely decreased kidney function (glomerular filtration rate <30 ml/min) had a 5.5-fold increased mortality risk within 2 weeks after an initial pulmonary embolism as compared with persons with a filtration rate ≥30 ml/min.[33] The investigation of non-fatal events and fatality rates was beyond our scope, since we focused on mortality rates. However, based on the higher prevalence of pulmonary embolism as a cause of death in dialysis patients (0.7%) than in the general population (0.5%), the 12.2-fold increased mortality risk due to pulmonary embolisms is probably not only a reflection of higher mortality rates in dialysis patients than in the general population.

Several explanations for an increased incidence of pulmonary embolism in dialysis patients are possible. An explanation could be the higher rate of hospitalization, surgery, and immobilization in dialysis patients than in the general population. Furthermore, several studies have demonstrated a hypercoagulable state in dialysis patients.[34,35] Hypercoagulability due to vasculitis could also explain the increased risk of pulmonary embolism in patients with multi-system disease, including patients with systemic lupus erythematosus or granulomatous polyangiitis,[36-38] while nephrotic-range proteinuria could explain the increased risk in patients with diabetes as both diabetes and pulmonary embolism have been associated with the nephrotic syndrome.[39] However, the nephrotic syndrome probably plays a less important role in the development of pulmonary embolisms in dialysis patients than in other kidney disease patients, since the nephrotic syndrome is less common in dialysis patients because

of the fast loss of residual renal function. Another plausible explanation for the increased risk of venous thrombosis in dialysis patients could be thrombus formation associated with catheters and arteriovenous accesses for dialysis which may cause pulmonary embolisms through dislodgement of thrombi.[40,41] We had no information about type of access in this study. Moreover, anticoagulation could be withheld in dialysis patients, because of the increased bleeding risk. We also had no information about anticoagulation use.

Several explanations for the highly increased mortality risk of myocardial infarction and stroke are possible. The high risk of myocardial infarction and stroke could be explained by the much higher prevalence of traditional risk factors for cardiovascular diseases, such as diabetes, hypertension, and left ventricular hypertrophy in the dialysis population than in the general population.[14,15,27,32,42] In addition, chronic kidney disease has been shown to be associated with inflammation and accelerated atherosclerotic vascular disease that subsequently could increase the mortality risk from myocardial infarction or ischemic stroke.[43,44] Moreover, anticoagulation of patients combined with uremic bleeding diathesis, may cause an increased risk of hemorrhagic stroke. Unfortunately, our data did not allow the calculation of mortality rate ratios for ischemic and hemorrhagic stroke separately.

A potential limitation of our study was that the cause of death was unknown in approximately 13.8% of the dialysis patients compared with 2.0% of the general population. This difference can be explained by the slightly different method for assigning the cause of death in dialysis patients as compared with the general population. For example, the autopsy is performed less commonly in dialysis patients than in the general population. Causes of death among patients on dialysis were recorded by the primary nephrologist. When a patient died at home or elsewhere outside the hospital, the nephrologist will have been dependent on information from others, and may more likely report a cause of death as unknown. Conversely, causes of death within the general population are, according to law, recorded by the physician who confirmed the death and thereafter sent the data to the statistics office, resulting in relatively fewer missing causes of death. Since the proportion of missing causes of death is greater in dialysis patients than in the general population, it is likely that this study underestimated the mortality rate ratios for total and specific cardiovascular mortality in dialysis patients as compared with the general population due to misclassification of cardiovascular death as unknown. Furthermore, misclassification may have become even worse due to the potential attribution of the code sudden cardiac death to dialysis patients who in reality may have died from pulmonary embolism, myocardial infarction, or stroke. In a highly unlikely worst case scenario, in which we change all unknown codes of death in the general population into pulmonary embolism, myocardial infarction, or stroke, we would still observe increased age- and sex-standardized mortality rate ratios for pulmonary embolism (2.7; 95% CI 2.3-3.3),

and also for myocardial infarction (8.4; 95% CI 8.0-8.8) and stroke (6.8; 95% CI 6.5-7.2), respectively.

In conclusion, dialysis patients have an unexpected highly increased mortality risk due to pulmonary embolism, and an increased mortality risk due to myocardial infarction, stroke, and other cardiovascular diseases as compared with the general population.

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Chapter 8

Hemodialysis catheters increase mortality as compared to arteriovenous accesses especially in elderly patients

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ABSTRACT

Background: Catheter use has been associated with an increased mortality risk in hemodialysis patients. However, differences in the all-cause and cause-specific mortality risk between catheter use and arteriovenous access use in young and elderly hemodialysis patients has not yet been investigated.

Methods: In this prospective cohort study of 1109 incident hemodialysis patients from 38 centers in the Netherlands, hazard ratios (HRs) with 95% confidence intervals (95% CIs) were calculated for 2-year all-cause, infection-related, and cardiovascular mortality in patients with a catheter as compared to patients with an arteriovenous access stratified for age (< 65 years and ≥ 65 years).

Results: Of the 1109 patients, 919 had an arteriovenous access and 190 had a catheter. The mortality rate was 76 per 1000 person-years in young patients with an arteriovenous access, 129 per 1000 person-years in young patients with a catheter, 222 per 1000 person-years in elderly patients with an arteriovenous access, and 427 per 1000 person-years in elderly patients with a catheter. The adjusted HR was 3.15 (95% CI 2.09-4.75) for elderly patients with a catheter as compared to young patients with an arteriovenous access. The adjusted HRs in elderly patients with a catheter as compared to elderly patients with an arteriovenous access were 1.54 (95% CI 1.13-2.12) for all-cause mortality, 1.60 (95% CI 0.62-4.19) for infection-related mortality, and 1.67 (95% CI 1.04-2.68) for cardiovascular mortality.

Conclusion: Especially elderly hemodialysis patients with a catheter have an increased all-cause, infection-related and cardiovascular mortality risk as compared to patients with an arteriovenous access.

INTRODUCTION

Dialysis patients require a vascular access for hemodialysis therapy. However, vascular access problems are responsible for 25% to 50% of hospitalizations in hemodialysis patients and are also associated with high costs.[1-6] While evidence from randomized-controlled trials is lacking, there is a broad consensus that arteriovenous accesses (fistula or graft) are superior to central venous catheters. Catheter use for hemodialysis has been associated with an increased risk for thrombosis,[7,8] short access survival,[8,9] and an increased risk for infections.[10-13] Therefore, the National Kidney Foundation Kidney Disease Outcome Quality Initiative (NKF K/DOQI) guidelines[14] and the European Best Practice Guidelines[15] recommend the use of arteriovenous accesses instead of catheters for vascular access in hemodialysis patients.

Despite this preference and recommendation for arteriovenous access use instead of catheters, limited studies have investigated the association between catheter use and mortality in elderly hemodialysis patients. Three studies from the United States have reported an increased mortality risk in elderly hemodialysis patients (elderly defined as age ≥ 65 years in two studies and aged ≥ 67 years in one study) ranging from a 1.3 to 2.1-fold increased risk for mortality in patients with a catheter as compared to patients with an arteriovenous access.[16-18] Moreover, information about differences in the association between catheter use and all-cause and cause-specific mortality in incident hemodialysis patients is limited and needs further exploration. This information is important, since the cause-specific and all-cause mortality risk could be different in elderly patients as compared to young patients, leading to age-specific treatment strategies.

Therefore, we investigated the association between catheter use versus arteriovenous access use and effect on all-cause and cause-specific (infection-related and cardiovascular) mortality risk in elderly hemodialysis patients as compared to young hemodialysis patients from a Dutch cohort of incident dialysis patients.

METHODS

Patients

The Netherlands Cooperative Study on the Adequacy of Dialysis (NECOSAD) is a prospective multicenter cohort study in which incident adult end-stage renal disease patients from 38 dialysis centers in the Netherlands were included.[19] All patients gave informed consent and the study was approved by all local medical ethics committees. We followed patients at three months and six months after start of dialysis and thereafter every six months until death

or censoring, i.e. transfer to a nonparticipating dialysis center, withdrawal from the study, transplantation, or end of the follow-up period (April 2006).

Eligibility included age older than 18 years, no previous renal replacement therapy, and survival of the initial three months of dialysis. For the current analyses, we used data from hemodialysis patients included between January 1997 and April 2004. The baseline was defined at three months after the start of dialysis. This time point of three months was chosen because patients' switch to another therapy or deaths within this period were most probably due to their health status before the start of dialysis, rather than to the dialysis modality.

Demographic and clinical data

Data on age, sex, primary kidney disease, comorbidity, predialysis care, diabetes, and cardiovascular disease (angina pectoris, myocardial infarction, heart failure, ischemic stroke, or claudication) were collected at the start of dialysis treatment. Primary kidney disease was classified according to the codes of the European Renal Association-European Dialysis and Transplant Association (ERA-EDTA).[20] We grouped patients into four classes of primary kidney disease: glomerulonephritis, diabetes mellitus, renal vascular disease, and other kidney diseases. Other kidney diseases consisted of patients with interstitial nephritis, polycystic kidney diseases, other multisystem diseases, and unknown diseases. The comorbidity was scored on the basis of the number comorbid conditions according to the comorbidity index described by Davies et al.[21]. The patients were classified as having no, intermediate, or severe comorbidity. Since comorbidity is an important confounder for the association between arteriovenous access use versus catheter use and mortality, the Davies score is used to adjust for comorbidity. The Davies score is based on the presence or absence of seven comorbid conditions, producing three risk groups. The Davies score assigns 1 point for each of the following conditions: ischemic heart disease (defined as prior myocardial infarction, angina pectoris, or ischemic changes on electrocardiogram), left ventricular dysfunction (defined as clinical evidence of pulmonary edema not due to errors in fluid balance), peripheral vascular disease (includes distal aortic, lower extremity, and cerebrovascular disease), malignancy, diabetes, collagen vascular disease, and other significant disorder (e.g. chronic obstructive pulmonary disease). Predialysis care was defined as a referral to a nephrologist for at least three months before initiation of dialysis to provide patients with adequate medical preparation.

Data on vascular access, Kt/Vurea delivered by hemodialysis, and body mass index (BMI) were collected at 3 months after the start of dialysis. Catheters included both tunneled and non-tunneled catheters (jugular and femoral) and arteriovenous accesses included native fistulas and grafts; data on native fistula and graft were not available, though. BMI was calculated as weight in kilograms divided by height in meters squared. The Kt/Vurea delivered

by hemodialysis was estimated according to the second-generation Daugirdas formula on the basis of one plasma urea measurement before and one immediately after the dialysis session, the ultrafiltration, and the duration of the session as described previously.[22,23] Blood and 24-hour urine samples were obtained at 3 months after the start of dialysis. Albumin, creatinine, urea, cholesterol, and C-reactive protein (CRP) were determined from the blood samples. Urea and creatinine levels were also measured in the urine samples. Renal function, expressed as glomerular filtration rate (GFR), was calculated as the mean of creatinine and urea clearance corrected for body surface area (ml/min per 1.73 m^2). GFR was missing in 250 patients and serum albumin in 35 patients. The missing values for GFR and serum albumin were imputed with multiple imputation, a recommended technique where missing data for a subject are imputed by a value that is predicted by using the subject's other, known characteristics,[24,25] i.e. using demographic characteristics, mortality, catheter use and serum albumin, creatinine, and GFR at different time points. We used standard imputation methods in SPSS statistical software (version 17.0; SPSS, Chicago, Illinois).

Outcome definition

The endpoint of this study was 2-year mortality. We classified causes of death according to the codes of the ERA-EDTA and grouped death causes into cardiovascular, infection-related, and other mortality. The following codes were designated as cardiovascular mortality: myocardial ischemia and infarction; cardiac failure/fluid overload/pulmonary edema; cardiac arrest, cause unknown; cerebrovascular accident; hemorrhage from ruptured vascular aneurysm; mesenteric infarction; hyperkalemia; hypokalemia; cause of death uncertain/unknown. The following codes were designated as infection-related mortality: pulmonary infection; infections elsewhere except viral hepatitis; septicaemia; tuberculosis; generalized viral infection; peritonitis. All other deaths were designated as other.

Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD) or as median and interquartile rage (IQR) depending on the normality of the data. Categorical variables are presented as number with valid percentages. For continuous data, differences for arteriovenous access use versus catheter use were tested with *t* test or Mann–Whitney–Wilcoxon test, depending on the distribution of the data. Chi-square test was used for categorical variables. A *P* value less than 0.05 was considered significant.

Survival curves were determined with the Kaplan-Meier method and mortality rates per 1000 person-years were calculated for four categories of hemodialysis patients defined by age group and vascular access (elderly arteriovenous access users aged ≥ 65 years, young arteriovenous access users aged < 65 years, elderly catheter users aged ≥ 65 years, and

young catheter users aged < 65 years). We calculated crude and adjusted hazard ratios (HRs) with 95% confidence intervals (95% CIs) for all-cause, infection-related, and cardiovascular mortality within 2-year of follow-up in young and elderly hemodialysis patients using Cox proportional hazard analysis. Furthermore, we calculated HRs for elderly catheter users aged ≥ 65 years, elderly arteriovenous access users aged ≥ 65 years, and young catheter users aged < 65 years as compared to young arteriovenous access users aged < 65 years. In an additional analysis, HRs for mortality were calculated for very old patients with a catheter aged ≥ 75 years as compared to very old patients with an arteriovenous access and as compared to young patients with an arteriovenous access aged < 65 years. HRs were adjusted for age, sex, primary kidney disease, Davies comorbidity score, predialysis care, GFR, CRP, cholesterol, BMI, serum albumin levels, and Kt/Vurea delivered by hemodialysis. The association between catheter use and mortality was studied with an intention-to-treat design since we were interested in the effect of initial vascular access on mortality. All analysis have been done in SPSS statistical software version 17.0; SPSS, Chicago, Illinois.

RESULTS

A total of 1109 patients were included between January 1997 and April 2004 and were treated with hemodialysis therapy at 3 months after start of dialysis in the NECOSAD. Of these patients, 190 (17.1%) had a catheter and 919 (82.9%) had an arteriovenous access for hemodialysis as vascular access. Table 1 shows the baseline characteristics of these patients. There were no differences between patients with a catheter or arteriovenous access according to age, sex, angina pectoris, myocardial infarction, ischemic stroke, claudication, diabetes, Davies comorbidity score, distribution of primary kidney disease, and Kt/Vurea delivered by hemodialysis. The patients with a catheter had a lower BMI, received less often predialysis care, had more often heart failure, had lower serum albumin levels, had lower cholesterol levels, had higher CRP levels, and had a lower GFR as compared to patients with an arteriovenous access ($p<0.05$ for all).

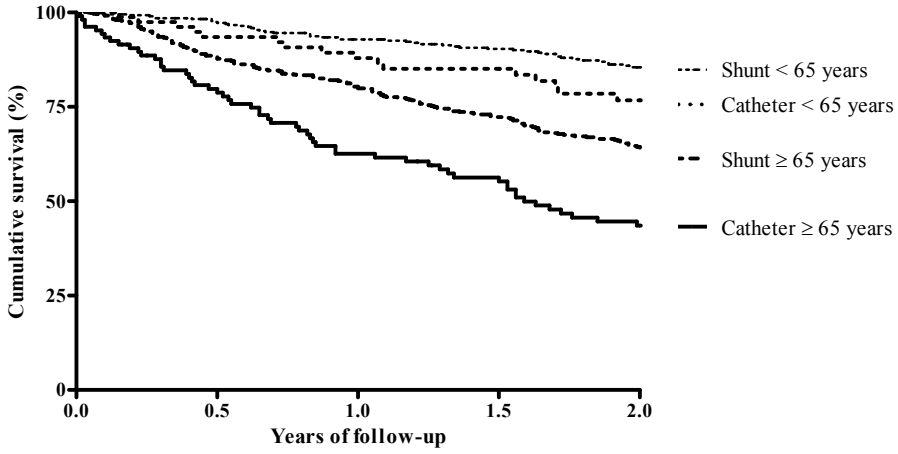
Table 1. Baseline characteristics

	Arteriovenous access N=919	Catheter N=190
Age (years) (%)	63.7 ± 13.6	63.2 ± 14.5
< 65 years	51.6 ± 10.6 (44.8)	50.1 ± 11.4 (44.2)
≥ 65 to 75 years	70.0 ± 2.9 (33.1)	70.1 ± 2.8 (33.2)
≥ 75 years	79.0 ± 3.1 (22.1)	78.8 ± 3.1 (22.6)
Sex, male (%)	58.7	55.8
BMI (kg/m^2)	24.7 ± 4.3	24.5± 4.5
Primary kidney disease (%)		
Diabetes mellitus	15.6	16.8
Glomerulonephritis	11.1	5.8
Renal vascular disease	21.0	27.4
Others	52.3	50.0
Cardiovascular disease (%)		
Angina pectoris	11.9	11.6
Myocardial infarction	13.5	15.3
Heart failure	12.1	20.5
Ischemic stroke	9.7	8.4
Claudication	16.2	17.9
Diabetes mellitus (%)	23.1	24.2
Davies comorbidity score (%)		
No	38.7	35.8
Intermediate	50.6	48.9
Severe	10.7	15.3
Predialysis care (%)	76.1	55.3
< 65 years	79.9	46.4
≥ 65 to 75 years	73.0	61.9
≥ 75 years	72.9	62.8
Serum albumin (g/L)	37.0 (33.3-40.0)	35.0 (32.0-38.0)
Cholesterol (mmol/L)	4.8 ± 1.2	4.5 ± 1.2
CRP (mg/L)	6.0 (3.0-12.5)	7.6 (3.0-13.0)
GFR (ml/min per 1.73 m^2)	3.1 (1.5-4.9)	2.9 (1.1-4.5)
Hemodialysis Kt/Vurea (week)	2.8 ± 0.8	2.8 ± 0.9

All-cause mortality

Of the 190 patients with a catheter, 72 patients died within two years. Of the 919 patients with an arteriovenous access, 217 died within two years. Figure 1 shows the Kaplan-Meier curves for all-cause mortality in young and elderly patients with an arteriovenous access or catheter for the first two years of follow-up. Table 2 shows the HRs for 2-year mortality for both age groups: young patients with a catheter as compared to young patients with an arteriovenous access and elderly patients with a catheter as compared to elderly patients with an arteriovenous access. The mortality rate was lowest for young arteriovenous access users and was highest for elderly catheter users. The HR for 2-year mortality was 1.54 (95% CI 0.87-2.74) in young

patients with a catheter as compared to young patients with an arteriovenous access after adjustment for age, sex, primary kidney disease, Davies comorbidity score, angina pectoris, myocardial infarction, heart failure, ischemic stroke, claudication, predialysis care, GFR, CRP, cholesterol, BMI, serum albumin levels, and Kt/Vurea delivered by hemodialysis. The adjusted HR for 2-year mortality was 1.54 (95% CI 1.13-2.12) in elderly patients with a catheter as compared to elderly patients with an arteriovenous access. However, on an absolute scale catheter use in elderly patients as compared to arteriovenous access use in elderly is associated with more deaths than catheter use in young patients as compared to arteriovenous access use in young patients (absolute risk difference of 205 per 1000 person-years versus 53 per 1000 person-years). As compared to young patients with an arteriovenous access, both young patients with a catheter and elderly patients with a catheter or arteriovenous access had an increased risk (Table 3); elderly patients with a catheter had an almost 6-fold crude and 3-fold adjusted increased mortality risk as compared to young patients with an arteriovenous access.



Number at risk:

Shunt < 65 years	412	371	320	273	235
Catheter < 65 years	84	70	62	54	42
Shunt ≥ 65 years	507	424	376	325	282
Catheter ≥ 65 years	106	80	61	53	41

Figure 1. Kaplan-Meier survival curve for arteriovenous access versus catheter in young and elderly hemodialysis patients

Table 2. All-cause mortality for catheter as compared to arteriovenous access in young and elderly hemodialysis patients

		Arteriovenous access (graft or fistula)	Catheter
Young < 65 years	Mortality rate per 1000 py	76	129
	Crude HR (95% CI)	1 (reference)	1.70 (0.97-2.99)
	Adjusted* HR (95% CI)	1 (reference)	1.74 (0.99-3.08)
	Adjusted† HR (95% CI)	1 (reference)	1.54 (0.87-2.74)
Elderly ≥ 65 years	Mortality rate per 1000 py	222	427
	Crude HR (95% CI)	1 (reference)	1.93 (95% CI 1.42-2.61)
	Adjusted* HR (95% CI)	1 (reference)	1.70 (95% CI 1.25-2.31)
	Adjusted† HR (95% CI)	1 (reference)	1.54 (95% CI 1.13-2.12)

py, person-years; HR, hazard ratio; CI, confidence interval. *Adjusted for age, sex, Davies comorbidity score, angina pectoris, myocardial infarction, heart failure, ischemic stroke, claudication, and primary kidney disease. †Additional adjusted for predialysis care, GFR, CRP, cholesterol, BMI, serum albumin levels, and hemodialysis Kt/Vurea.

Table 3. All-cause mortality for young and elderly patients with a catheter or arteriovenous access

	Mortality rate per 1000 person-years	Crude HR (95% CI)	Adjusted* HR (95% CI)	Adjusted† HR (95% CI)
Arteriovenous access < 65 years	76	1 (reference)	1 (reference)	1 (reference)
Catheter < 65 years	129	1.70 (0.97-2.99)	1.71 (0.97-3.02)	1.49 (0.84-2.66)
Arteriovenous access ≥ 65 years	222	2.93 (2.13-4.02)	2.12 (1.53-2.94)	2.06 (1.48-2.86)
Catheter ≥ 65 years	427	5.64 (3.84-8.27)	3.55 (2.39-5.28)	3.15 (2.09-4.75)

HR, hazard ratio; CI, confidence interval. *Adjusted for sex, Davies comorbidity score, angina pectoris, myocardial infarction, heart failure, ischemic stroke, claudication, and primary kidney disease. †Additional adjusted for predialysis care, GFR, CRP, cholesterol, BMI, serum albumin levels, and hemodialysis Kt/Vurea.

Cause-specific mortality

Of the 72 patients with a catheter who died within the first two years of follow-up, 7 died because of infections (1 young and 6 elderly patients), 34 because of cardiovascular causes (9 young and 25 elderly patients), and 31 died because of other reasons (6 young and 25 elderly patients). Of the 217 patients with an arteriovenous access who died within 2 years of follow-up, 27 died because of infections (7 young and 20 elderly patients), 103 because of cardiovascular causes (25 young and 78 elderly patients), and 87 died because of other reasons (17 young and 70 elderly patients). Figure 2 shows the adjusted HRs for cause-specific 2-year mortality in young and elderly patients. The adjusted HRs in elderly patients with a catheter were 1.60 (95% CI 0.62-4.19) for infection-related mortality and 1.67 (95% CI 1.04-2.68) for cardiovascular mortality as compared to elderly patients with an arteriovenous access. HRs in elderly patients with a catheter as compared to young patients with an arteriovenous access

were 2.92 (95% CI 0.91-9.37) for infection-related mortality and 3.09 (95% CI 1.70-5.60) for cardiovascular mortality after adjustment for sex, primary kidney disease, Davies comorbidity score, angina pectoris, myocardial infarction, heart failure, ischemic stroke, claudication, predialysis care, GFR, CRP, cholesterol, BMI, serum albumin levels, and Kt/Vurea delivered by hemodialysis.

Very old patients

There were 43 very old (aged≥ 75 years) patients with a catheter, 26 patients of whom died within two years (11 cardiovascular causes, 3 infection-related causes, and 12 other causes). Furthermore, 72 patients of the 203 very old patients with an arteriovenous access died within two years (32 cardiovascular causes, 8 infection-related causes, and 32 other causes). The mortality rate per 1000 person-years was 244 for very old arteriovenous access users and 505 for very old catheter users. The adjusted HRs in very old patients were 1.83 (95% CI 1.14-2.93) for all-cause mortality, 2.32 (95% CI 0.57-9.40) for infection-related mortality, and 1.96 (95% CI 0.96-4.02) for cardiovascular mortality as compared to very old patients with an arteriovenous access.

Figure 2. Adjusted hazard ratios with 95% confidence intervals for all-cause and cause-specific mortality for catheter as compared to arteriovenous access in young and elderly hemodialysis patients

Hazard ratios are adjusted for age, sex, Davies comorbidity score, angina pectoris, myocardial infarction, heart failure, ischemic stroke, claudication, primary kidney disease, predialysis care, GFR, CRP, cholesterol, BMI, serum albumin levels, and hemodialysis Kt/Vurea.

DISCUSSION

In this prospective cohort study of incident dialysis patients, we showed that catheter use was associated with an increased 2-year all-cause mortality risk as compared to arteriovenous access use. Elderly patients (aged ≥ 65 years) with a catheter had a 54% increased risk for mortality within 2 years as compared to elderly patients with an arteriovenous access and had a 3-fold increased risk for mortality within 2 years as compared to young patients (aged < 65 years) with an arteriovenous access. Very old patients with a catheter (aged ≥ 75 years) had an 83% increased mortality risk as compared to very old patients with an arteriovenous access. Among elderly patients, catheter use increased especially infection-related and cardiovascular mortality as compared to arteriovenous access use. The occurrence of septicemia or bacteremia has been shown to be associated with subsequent cardiovascular morbidity and mortality.[26] Our findings provide support to the guidelines which indicate that the use of catheters for hemodialysis should be discouraged. We showed that, especially in elderly hemodialysis patients, catheter use should be discouraged, since older age and catheter use are associated with an even higher increased mortality risk.

The 1.54-fold increased risk for mortality within two years of follow-up in elderly hemodialysis patients in our Dutch cohort is comparable to the increased risk found in cohorts from the United States. Three studies have reported a 1.3- to 2.1-fold increased risk for mortality in elderly patients with a catheter as compared to elderly patients with an arteriovenous access.[16-18] The first study used data from the United States Medicare dialysis population from 1995 to 1997.[16] They showed that catheter use was associated with a 1.7-fold increased one-year mortality as compared to native fistula use in elderly dialysis patients aged ≥ 67 years. The second study used data from the United States Renal Data System.[17] They showed in a subgroup analysis that catheter use was associated with a 2.1-fold increased mortality as compared to native fistula use in elderly dialysis patients aged ≥ 65 years over a mean follow-up of one year. Finally, the Choices for Healthy Outcomes in Caring for End-stage renal disease study showed in a subgroup analysis that catheter use was associated with a 1.3-fold increased mortality as compared to native fistula use in elderly dialysis patients aged ≥ 65 years over a follow-up of three years.[18]

Previous studies have reported that catheter use is associated with sepsis and bacteremia,[10-13] with decreased delivered dose of dialysis due to decreased blood flow rates,[27] and higher levels of inflammatory factors.[28] In addition, several studies have shown an increased infection-related and cardiovascular mortality risk in patients with a catheter as vascular access, especially in the first three months of dialysis.[29,30] However, to our knowledge, there are no studies that investigated infection-related or cardiovascular mortality associated with catheter

use in elderly patients. We showed that elderly patients with a catheter had a 60% increased risk for infection-related mortality and a 67% increased risk for cardiovascular mortality as compared to elderly patients with an arteriovenous access. Including the first three months of dialysis would probably have led to an even higher infection-related mortality. Furthermore, we showed that very old patients with a catheter had a 2.3-fold increased risk for infection-related mortality and a 2.0-fold increased risk for cardiovascular mortality as compared to very old patients with an arteriovenous access. The confidence intervals for the HR for the infection-related 2-year mortality were wide due to a low number of patients with infection-related mortality.

Missing values for GFR and serum albumin were imputed. Patients with missing GFR or serum albumin used more often catheters and had relatively higher mortality rates. Analyses excluding patients who had a missing GFR or serum albumin values would have led to biased results, since relative more patients with a catheter with higher mortality rates would have been excluded.[24] This would have resulted in a decreased mortality risk for catheter use as compared to arteriovenous access use. Therefore, imputation of GFR and serum albumin using demographic characteristics, mortality, catheter use, and creatinine, serum albumin and GFR at different time points leads to more reliable results.

The comparison between catheter use and arteriovenous access use in an observational design makes confounding-by-indication the most important obstacle. It is important to realize that these observational studies have limitations to prove causality, since the observed increased mortality risk in patients with a catheter may partly reflect the effect of differences between arteriovenous access and catheter users. Catheter use has been associated with less predialysis care, lower GFR, lower serum albumin levels, and more co-morbidity as compared to arteriovenous access users.[31-33] In our analyses, we took this into account by correcting for these confounders, but this cannot exclude possible residual confounding. Therefore, our study shows that catheter use is associated with an increased mortality risk, but this does not necessarily prove that catheter use increases mortality risk. However, even additional adjustment for diabetes, angina pectoris, myocardial infarction, heart failure, ischemic stroke, and claudication did not change the results. Furthermore, we compared catheter use with arteriovenous access use and not with native fistula use since this information was lacking in our study. Therefore, as according to the literature mortality is higher in patients with a graft compared to patients with a native fistula,[18,30] patients with catheters in our study would have had even higher relative mortality risks. Moreover, we had no information about the type of catheters (tunneled or non-tunneled), the insertion place of the catheters, and the use of antimicrobial locks for catheters to investigate differences in mortality risk in patients with a catheter. An intention-to-treat analysis was chosen for the analyses, because we

were interested in the association between mortality and initial vascular access treatment in elderly dialysis patients. This is important, since guidelines especially discourage catheter use as initial vascular access treatment.[14,15] In addition, an intention-to-treat analysis also avoids bias caused by transferring patients with an arteriovenous access with complications to the catheter group. An as-treated design would therefore overestimate the mortality-risk for patients with a catheter.

In conclusion, our study shows that catheter compared to arteriovenous access use is associated with an increased mortality, especially among elderly patients. Our findings are consistent with the guidelines which indicate that the use of catheters for hemodialysis should be discouraged. We showed that this is especially true for the elderly hemodialysis population.

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Chapter 9

Type of arteriovenous vascular access and association with patency and mortality

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ABSTRACT

Background: There are only a few risk factors known for primary patency loss in patients with an arteriovenous graft or fistula. Furthermore, a limited number of studies have investigated the association between arteriovenous access modality and primary patency loss and mortality. The aim of this study was to investigate risk factors for patency loss and to investigate the association between graft versus fistula use and outcomes (patency loss and mortality).

Methods: We prospectively followed 919 incident hemodialysis patients and calculated hazard ratios (HRs) for putative risk factors of primary patency loss using Cox regression. Furthermore, HRs were calculated to study the association between graft versus fistula use and two-year primary patency loss and two-year mortality.

Results: Cardiovascular disease, prior catheter use, lowest tertile of albumin, highest tertile of hsCRP, and lowest tertile of fetuin-A were associated with primary patency loss in both patients with grafts and fistulas. Increased age, female sex, and diabetes mellitus were only associated with primary patency loss in patients with a fistula. We did not observe an association between primary patency loss and BMI, residual GFR, levels of calcium, phosphorus, and total cholesterol. Furthermore, graft use as compared with fistula use was associated with an 1.4-fold (95%CI 1.0-1.9) increased risk of primary patency loss and with an 1.5-fold (95%CI 1.0-2.2) increased mortality risk.

Conclusion: Cardiovascular disease, prior catheter use, albumin, hsCRP, and fetuin-A are risk factors for patency loss. Graft use as compared with fistula use was associated with an increased risk of patency loss and mortality.

INTRODUCTION

Preservation of adequate vascular access is of vital importance for patients undergoing chronic hemodialysis. Vascular access-related morbidity accounts for 20% of all hospitalizations in hemodialysis patients leading to high costs.[1,2]

Several studies have shown that graft use as compared with fistula use was associated with an increased risk of patency loss.[3] However, few studies have investigated whether risk factors for patency loss are different for fistula use and graft use. The vast majority of arteriovenous access failure is caused by thrombosis, secondary to disproportionate intimal hyperplasia in the venous outflow tract.[4-7] The mechanisms that are responsible for this localized hyperplastic response are incompletely understood. The prevailing opinion is that local hemodynamic factors such as flow turbulence, vascular inflammation as well as the prothrombotic milieu that results from endothelial damage play a role in the formation of stenotic lesions.[8,9] Factors associated with atherosclerotic vascular disease and inflammation might play a different role in the formation of stenotic lesions in fistulas and graft. CRP and fetuin-A are both markers for inflammation and cardiovascular disease that could be associated with patency loss.

Moreover, a limited number of studies have investigated the association between fistula use versus graft use and mortality.[10,11] These studies found a moderately increased mortality risk for graft use as compared with fistula use.[10,11] The National Kidney Foundation Kidney Disease Outcome Quality Initiative guidelines[12] and the European Best Practice Guidelines[13] recommend the use of a fistula instead of grafts for vascular access in all hemodialysis patients. To date, it is unknown whether the graft use versus fistula use is associated with both patency loss and mortality.

In the present study, we investigated risk factors for primary patency loss (i.e. any intervention in the arteriovenous access after the first successful cannulation) in a large Dutch cohort of 919 incident hemodialysis patients.[14] In addition, we investigated the association between graft use versus fistula use and two-year patency loss and mortality.

METHODS

Patients

The Netherlands Cooperative Study on the Adequacy of Dialysis (NECOSAD) is a prospective multicenter cohort study in which incident adult end-stage renal disease patients from 38 dialysis centers in the Netherlands were included. The study was approved by all local medical ethics committees (Maasstad Hospital Rotterdam, Deventer Hospital Deventer,

Sint Lucas Andreas Hospital Amsterdam, Dianet Dialysis Center Academic Medical Center Amsterdam, Maxima Medical Center Veldhoven, Catharina Hospital Eindhoven, Medical Center Haaglanden Den Haag, University Medical Center Groningen, Kennemer Gasthuis Haarlem, Atrium Medical Center Heerlen, Medical Center Leeuwarden, Leiden University Medical Center Leiden, Elisabeth Hospital Tilburg, University Medical Center Utrecht, Antonius Ziekenhuis Nieuwegein, Hospital Gelderse Vallei Ede, Haga Hospital Leyenburg Den Haag, Academic Hospital Maastricht, Jeroen Bosch Hospital Den Bosch, Medisch Spectrum Twente Enschede, Albert Schweitzer Hospital Dordrecht, Alysis Zorggroep Rijnstate Hospital Arnhem, Dianet Dialysis Center Lunetten Utrecht, Canisius Wilhelmina Hospital Nijmegen, Vie Curi Medical Center Venlo, Leveste Scheper Hospital Emmen, Dianet Dialysis Center Holendrecht Amsterdam, Hage Hospital Rode Kruis Den Haag, Rijnland Hospital Leiderdorp, Admiraal de Ruyter ziekenhuis Goes, Medical Center Alkmaar, Laurentius Ziekenhuis Roermond, Dialysis Center 't Gooi Hilversum, Groene Hart Hospital Gouda, Westfries Gasthuis Hoorn, Tergooiziekenhuizen Hospital Hilversum, Martini Ziekenhuis Groningen, Zaans Medical Center Zaandam). We followed patients until death or censoring, i.e. transfer to a nonparticipating dialysis center, withdrawal from the study, transplantation, or end of the follow-up period (April 2006). We did not censor for patency loss when investigating the effect of fistula versus graft use on mortality.

Eligibility included age older than 18 years, no previous renal replacement therapy, and survival of the initial three months of dialysis. For the current analyses, we used data from incident hemodialysis patients included between January 1997 and April 2004 with a functional arteriovenous access (native fistulas or grafts) within three months after the first dialysis session. Information about graft use or fistula use was collected from the medical records of patients. All patients gave informed consent.

Demographic and clinical data

Data on age, sex, body mass index (BMI), primary kidney disease, cardiovascular disease (angina pectoris, myocardial infarction, heart failure, ischemic stroke, or claudication), and diabetes mellitus were collected at the start of dialysis treatment. BMI was calculated as weight in kilograms divided by height in meters squared. Primary kidney disease was classified according to the codes of the European Renal Association-European Dialysis and Transplant Association (ERA-EDTA).[15] We grouped patients into four classes of primary kidney disease: glomerulonephritis, diabetes mellitus, renal vascular disease, and other kidney diseases. Other kidney diseases consisted of patients with interstitial nephritis, polycystic kidney diseases, other multisystem diseases, and unknown diseases.

The following biochemical parameters were routinely measured in blood samples obtained from patients at 3 months after the start of dialysis: creatinine, urea, calcium, phosphorus, albumin, total cholesterol. Renal function, expressed as glomerular filtration rate (GFR), was calculated as the mean of creatinine and urea clearance corrected for body surface area (ml/min per 1.73 m^2). Moreover, circulating fetuin-A serum levels and high-sensitivity C-reactive protein (hsCRP) were measured at 3 months after the start of dialysis as described elsewhere.[16]

Primary patency loss and mortality

Time to primary patency loss was defined as the interval from time of first successful cannulation of the vascular access for hemodialysis treatment (first dialysis session) to surgery, percutaneous endovascular intervention, or abandonment of the vascular access in the first two years on dialysis. Information about surgery, percutaneous endovascular intervention, or abandonment of the vascular access was obtained from the standard data collection of NECOSAD. Two-year mortality was recorded according to the codes of the ERA-EDTA.[15]

Statistical analysis

Continuous variables are presented as median and interquartile range (IQR). Categorical variables are presented as number with percentages. We calculated hazard ratios (HRs) with 95% confidence intervals (95% CIs) using Cox regression analysis. HRs were calculated for primary patency loss within two-years of follow-up for previous catheter use, factors associated with atherosclerotic vascular disease (age, sex, diabetes mellitus, body mass index, residual glomerular filtration rate (GFR), calcium levels corrected for albumin, phosphorus levels, cholesterol levels, and fetuin-A levels), and factors associated with inflammation (hsCRP and albumin) stratified for patients with a fistula and patients with a graft use. Confounders were defined as variables that could be associated with exposure and with outcome, based on previous literature, without being an intermediate variable in the causal pathway between exposure and outcome.[17] Therefore, each investigated variable could have a different set of variables that were adjusted for. For the same reason, the effect of sex was not adjusted for other variables.

Survival curves for primary patency loss and mortality were determined with the Kaplan–Meier method stratified for fistula use and graft use. We calculated crude and adjusted hazard ratios (HRs) with 95% confidence intervals (95% CIs) for primary patency loss and mortality within 2 years of follow-up for patients with a fistula and patients with a graft using Cox regression analysis. All analyses have been done in SPSS statistical software version 18.0 (SPSS, Chicago, Ill).

RESULTS

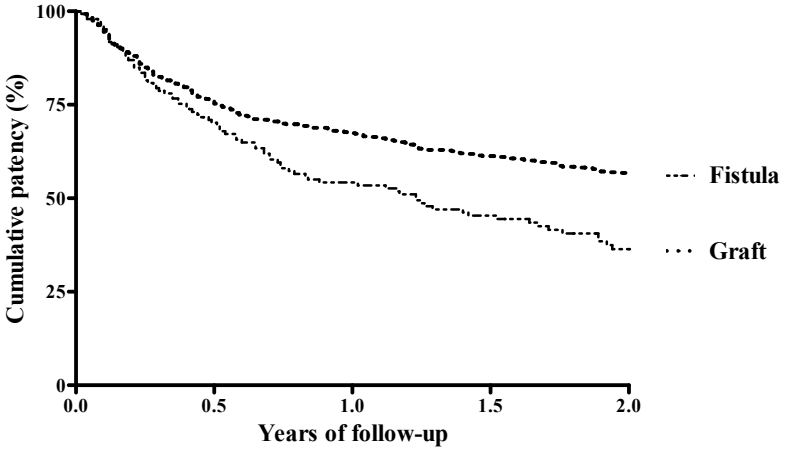
A total of 919 incident hemodialysis patients with an arteriovenous access were included in NECOSAD. Of the 919 patients, 727 had a fistula, 146 had a graft, and 46 patients had an unknown type of arteriovenous access. Patients with a graft were older, were more often female, had a higher body mass index, had more often diabetes mellitus as primary kidney disease, had lower residual GFR and had higher total cholesterol levels than patients with a fistula (P<0.05) (Table 1).

Table 1. Baseline characteristics of patients with a fistula and graft

	Fistula N=727		Graft N=146	
Age (years) (IQR)	65.8	(54.5-73.7)	68.5	(59.4-74.3)
Sex, female (%)	37.4		59.6	
BMI (kg/m^2) (IQR)	24.0	(22.0-26.7)	24.8	(21.5-27.8)
Primary kidney disease (%)				
Diabetes mellitus	14.7		17.8	
Glomerulonephritis	12.0		5.5	
Renal vascular disease	19.1		28.1	
Others	54.2		48.6	
Cardiovascular disease (%)	40.0		42.5	
Prior catheter use (%)	26.8		32.2	
Systolic blood pressure, mmHg	148	(135-162)	144	(133-158)
GFR (ml/min/1.73 m^2) (IQR)	3.2	(1.7-5.0)	2.4	(1.1-4.4)
Calcium (mmol/L) (IQR)	2.4	(2.2-2.5)	2.4	(2.2-2.6)
Phosphorus (mmol/L) (IQR)	1.8	(1.5-2.2)	1.8	(1.5-2.2)
Serum cholesterol (mmol/L) (IQR)	4.6	(3.9-5.4)	4.9	(4.1-6.0)
Serum albumin (g/L) (IQR)	37	(34-40)	36	(32-40)
hsCRP* (mg/L) (IQR)	5.3	(2.0-13.8)	6.5	(2.5-16.7)
Fetuin-A* (g/L) (IQR)	0.6	(0.5-0.7)	0.6	(0.5-0.8)

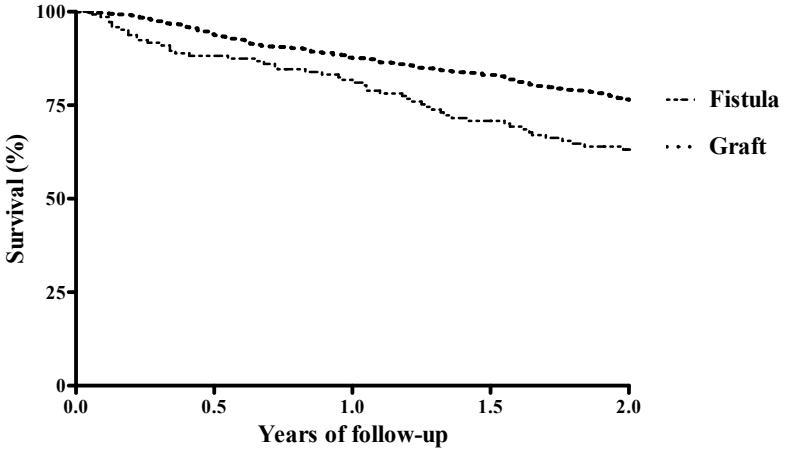
BMI, body mas index; IQR, interquartile range. *Missing in 276 patients with a fistula and 63 patients with a graft

During the two-year follow-up, 287 of the 727 patients with a fistula and 84 of the 146 patients with a graft lost primary patency of their vascular access within two years. The cumulative incidence at two years for primary patency at two years was 56.8% for patients with a fistula and was 36.4% for patients with a graft (Figure 1). Of the 727 patients with a fistula, 149 died within 2 years. Of the 146 patients with a graft, 51 died within 2 years. Figure 2 shows the Kaplan-Meier survival curve with two-years mortality as outcome for patients with a fistula and graft. The cumulative survival was 76.4% for patients with a fistula and 63.2% for patients with a graft.



Number at risk:					
Graft	146	96	71	54	34
Fistula	727	532	414	322	261

Figure 1. Kaplan-Meier survival curve for two-year primary patency loss after first successful cannulation



Number at risk:					
Graft	146	125	114	94	79
Fistula	727	643	554	481	418

Figure 2. Kaplan-Meier survival curve for two-year mortality

Table 2 shows the adjusted hazard ratios of the risk factors for primary patency loss stratified for patients with a fistula and patients with graft. Increased age ≥65 years (HR 1.3; 95% CI 1.0-1.7), female sex (HR 1.5; 95% CI 1.2-2.9), and diabetes mellitus (HR 2.0; 1.4-2.7) were associated with an increased risk of primary patency loss for patients with a fistula and not for patients with a graft. Cardiovascular disease (HR 1.7; 95% CI 1.3-2.2 and HR 1.8; 95% CI 1.1-2.9), prior catheter use (HR 1.9; 95% CI 1.5-2.4 and HR 2.1; 95% CI 1.3-3.4), lowest tertile of albumin (HR 1.5; 95% CI 1.1-2.1 and HR 2.4; 95% CI 1.3-4.5), highest tertile of hsCRP (HR 1.6; 95% CI 1.1-2.3 and HR 2.7; 95% CI 1.2-6.3), and lowest tertile of fetuin-A (HR 1.9; 95% CI 1.3-2.9 and HR 3.6; 95% CI 1.7-7.4) were associated with primary patency loss after adjustment in both patients with a fistula and graft, respectively. We did not find an association between primary patency loss and BMI, GFR, levels of calcium corrected for albumin, phosphorus, and cholesterol after adjustment (Table 2).

Graft use as compared with fistula use was associated with an 1.4-fold (95% CI 1.0-1.9) increased risk of primary patency loss and with an 1.5-fold (95% CI 1.0-2.2) increased two-year mortality risk after adjustment for age, sex, BMI, primary kidney disease, cardiovascular disease, prior catheter use, and levels of calcium, phosphorus, and cholesterol (Table 3).

Table 2. Risk factors for primary patency loss

			Fistula N=727 hazard ratio (95% CI)		Graft N=146 hazard ratio (95% CI)	
			Crude	Adjusted	Crude	Adjusted
Age* (years)	≥65 versus <65		1.3 (1.1-1.7)	1.3 (1.0-1.7)	1.1 (0.7-1.7)	1.1 (0.7-1.7)
Sex†	Female versus Male		1.5 (1.2-2.9)	1.5 (1.2-1.9)	0.7 (0.5-1.1)	0.7 (0.5-1.1)
BMI‡ (kg/m^2)	≥25 versus <25		1.0 (0.8-1.2)	0.9 (0.7-1.1)	0.8 (0.5-1.3)	0.8 (0.5-1.3)
Primary kidney disease§	Diabetes mellitus		2.1 (1.6-2.9)	2.0 (1.4-2.7)	1.4 (0.8-2.5)	1.2 (0.7-2.1)
	Glomerulonephritis		0.8 (0.6-1.3)	0.9 (0.6-1.3)	1.1 (0.4-3.1)	1.2 (0.4-3.5)
	Vascular disease		1.0 (0.8-1.4)	0.9 (0.7-1.3)	0.9 (0.5-1.5)	0.7 (0.4-1.2)
	Others		1 (reference)	1 (reference)	1 (reference)	1 (reference)
Cardiovascular disease¶	Yes versus No		1.7 (1.4-2.2)	1.7 (1.3-2.2)	1.8 (1.2-2.8)	1.8 (1.1-2.9)
Prior catheter use**	Yes versus No		1.9 (1.5-2.4)	1.9 (1.5-2.4)	2.2 (1.4-3.4)	2.1 (1.3-3.4)
Systolic blood pressure**	Low	<139	0.9 (0.7-1.2)	0.9 (0.7-1.2)	1.7 (1.0-2.9)	1.5 (0.8-2.6)
(mmHg)	Median	139-155	1.0 (0.8-1.4)	1.1 (0.8-1.4)	1.0 (0.6-1.8)	1.1 (0.6-2.0)
	High	>155	1 (reference)	1 (reference)	1 (reference)	1 (reference)
GFR**	>10		1 (reference)	1 (reference)	1 (reference)	1 (reference)
(ml/min/1.73 m^2)	5-10		1.1 (0.5-2.3)	1.0 (0.5-2.1)	0.5 (0.1-1.6)	0.6 (0.2-2.1)
	<5		0.9 (0.5-1.9)	0.9 (0.4-1.7)	0.4 (0.1-1.3)	0.4 (0.1-1.4)
Calcium** (mmol/L),	Low	<2.30	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Tertiles	Median	2.30-2.49	1.3 (0.9-1.7)	1.3 (1.0-1.7)	1.0 (0.6-1.8)	1.1 (0.6-1.9)
	High	>2.49	1.0 (0.8-1.4)	1.0 (0.8-1.4)	1.4 (0.8-2.3)	1.5 (0.9-2.5)
Phosphorus** (mmol/L),	Low	<1.57	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Tertiles	Median	1.57-2.02	0.9 (0.7-1.2)	0.9 (0.7-1.2)	0.7 (0.4-1.2)	0.8 (0.4-1.3)
	High	>2.02	0.9 (0.7-1.2)	0.9 (0.7-1.2)	0.9 (0.5-1.5)	0.9 (0.5-1.5)
Cholesterol** (mmol/L),	Low	<4.20	1 (reference)	1 reference)	1 (reference)	1 (reference)
Tertiles	Median	4.20-5.10	0.7 (0.5-0.9)	0.7 (0.5-1.0)	0.9 (0.5-1.6)	0.9 (0.5-1.6)
	High	>5.10	0.8 (0.6-1.1)	0.8 (0.6-1.1)	0.7 (0.4-1.2)	1.0 (0.5-1.7)
Albumin** (g/L),	Low	<35.0	1.8 (1.3-2.4)	1.5 (1.1-2.1)	3.1 (1.7-5.5)	2.4 (1.3-4.5)
Tertiles	Median	35.0-38.9	1.3 (1.0-1.8)	1.2 (0.9-1.7)	2.1 (1.1-4.0)	2.4 (1.2-4.6)
	High	>38.9	1 (reference)	1 (reference)	1 (reference)	1 (reference)
hsCRP** (mg/L),	Low	<2.95	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Tertiles	Median	2.95-9.94	1.1 (0.7-1.6)	1.1 (0.7-1.6)	2.5 (1.1-5.5)	2.6 (1.0-6.5)
	High	>9.94	1.6 (1.1-2.4)	1.6 (1.1-2.3)	2.5 (1.1-5.6)	2.7 (1.2-6.3)
Fetuin-A** (g/L),	Low	<0.55	1.9 (1.3-2.9)	1.9 (1.3-2.9)	3.6 (1.4-6.7)	3.6 (1.7-7.4)
Tertiles	Median	0.55-0.64	1.5 (1.0-2.3)	1.5 (1.0-2.3)	3.1 (1.8-7.3)	3.3 (1.5-7.5)
	High	>0.64	1 (reference)	1 (reference)	1 (reference)	1 (reference)

*Adjusted for sex, †Unadjusted, ‡Adjusted for age, sex, primary kidney disease, and cardiovascular disease, §Adjusted for age, sex, BMI, and cardiovascular disease, ¶Adjusted for age, sex, BMI, and primary kidney disease, **Adjusted for age, sex, BMI, primary kidney disease, and cardiovascular disease

Table 3. Association between graft versus fistula and patency loss and mortality

	Type of access			Patency loss HR (95% CI)	Mortality HR (95% CI)
Total	Fistula	(N=727)		1 (reference)	1 (reference)
	Graft	(N=146)	Crude	1.6 (1.3-2.0)	1.7 (1.3-2.4)
			Adjusted*	1.4 (0.9 -2.1)	1.5 (1.0-2.2)

HR, hazard ratio; CI, confidence interval. *Adjusted for age, sex, BMI, primary kidney disease, cardiovascular disease, prior catheter use, GFR, calcium corrected for albumin, phosphorus, and cholesterol

DISCUSSION

In this prospective cohort study of 919 incident hemodialysis patients with an arteriovenous access, we showed that cardiovascular disease, prior catheter use, levels of albumin, hsCRP, and fetuin-A were associated with primary patency loss in both patients with a fistula and a graft. Increased age, female sex, and diabetes mellitus was only associated with an increased risk of primary patency loss in patients with a fistula. We did not find an association between primary patency loss and BMI, GFR, and levels of calcium, phosphorus, and cholesterol. Furthermore, we showed that graft use as compared with fistula use was associated with an 1.4-fold (95% CI 1.0-1.9) increased risk of primary patency loss and with an 1.5-fold (95% CI 1.0-2.2) increased two-year mortality risk.

Previous smaller studies on risk factors of arteriovenous dysfunction have shown conflicting results.[18-25] Increased age has been associated in previous studies with vascular access morbidity,[18,19,21] but not in other studies.[20,22,23] Similar inconsistencies have been observed in previous studies on gender as a risk factor for arteriovenous access dysfunction.[20-23,25] In our large study using incident hemodialysis patients with an arteriovenous access, we have showed that increased age, female gender, and diabetes mellitus were associated with primary patency loss in patients with a fistula and not in patients with a graft. Since patients with a graft are a group of selected dialysis patients with an increased mortality risk and an increased risk of patency loss as compared with patients with a fistula, selection bias could explain the differences in the association between patency loss and age, sex, and presence of diabetes mellitus. Another reason could be that graft patency is less influenced by age, sex, and diabetes mellitus than fistula patency. Furthermore, we had less power in the graft group than in the fistula group for the investigation of risk factors for patency loss. In line with our study, cardiovascular disease[18,21] and prior catheter use[24] have been shown to be important risk factors for arteriovenous access dysfunction in other studies.

Limited studies have investigated the association between arteriovenous access dysfunction and BMI, GFR, calcium, phosphorus, and cholesterol. In concordance with the results of previous studies,[19-21] these potential risk factors were not associated with arteriovenous access dysfunction in our study. Fetuin-A, hsCRP, and albumin levels have been associated with mortality in dialysis patients.[25-28] However, a new observation in our study was that levels of fetuin-A, hsCRP, and albumin levels were associated with vascular access dysfunction in patients with a fistula and in patients with a graft.

The pathogenic mechanisms for arteriovenous dysfunction are incompletely understood, but it is thought that thrombosis resulting from stenosis due to neointimal hyperplasia is the main cause of arteriovenous dysfunction.[7-9] The stimuli responsible for this localized intimal hyperplastic response in the venous outflow tract are multifactorial and include hemodynamic factors such as turbulent flow, endothelial damage as well as repetitive strain injury and vascular inflammation that might relate to compliance mismatch between the anastomosed blood vessels.[7-9] The stenotic vascular lesions that arise from this intimal hyperplastic response mainly consist of vascular smooth muscle cells, myofibroblasts and extracellular matrix proteins such as collagen.[7-9] A recent study showed that the stenotic vascular lesions are already present prior to dialysis access placement.[29] Morphologically, these lesions differ substantially from atherosclerotic lesions that mainly consist of lipid-rich foam cells and activated T-cells [30,31]. Interestingly, our study suggests that well-known risk factors for atherosclerosis (cardiovascular disease and fetuin-A levels) and factors associated with inflammation (C-reactive protein and albumin) might play an important role in the development of stenotic lesions in arteriovenous fistulas in dialysis patients as well.

In the present study, graft use as compared with fistula use was associated with an 1.4-fold (95% CI 1.0-1.9) increased risk of primary patency loss and with an 1.5-fold (95% CI 1.0-2.2) increased two-year mortality risk. Previous studies found also an increased risk of patency los in patients with a graft as compared with patients with a fistula. However, limited studies have investigated the association between type of arteriovenous access and mortality.[10,11] These studies suggested an increased mortality risk for graft use as compared with fistula use.[10,11] Although National Kidney Foundation Kidney Disease Outcome Quality Initiative guidelines[12] and the European Best Practice Guidelines[13] recommend the use of a fistulas instead of grafts for vascular access in all hemodialysis patients, it could be that for special subgroups, such as elderly patients, grafts are good alternatives as first option for a vascular access, especially when we take into account that failure of vascular access before successful cannulation for dialysis is higher for fistulas than for grafts.[32]

Our study has several potential limitations. We had no information on several vascular access characteristics (anatomic location, flow, vessel diameter, and intervention prior to cannulation) that are associated with vascular access dysfunction. Moreover, type of arteriovenous access was unknown in 46 patients. However, when unknown type of vascular access was either classified as graft use or fistula, the influence of these unknown type of vascular accesses in the association between graft use versus fistula use and patency loss or mortality was minimal (data not shown). Another limitation is that confounding-by-indication could occur when comparing different outcomes for graft use versus fistula use in an observational design. The observed increased mortality risk and patency loss of graft use versus fistula use may partly reflect the effect of other differences between graft and fistula users. In our analyses, we took this into account by correcting for many confounders, but this cannot exclude possible residual confounding. Therefore, randomized controlled trials are needed when comparing outcomes between graft use and fistula use. However, there might be ethical and practical problems to conduct this kind of a randomized controlled trial. In view of the clinical importance in combination with the small differences in outcomes between graft use and fistula use in elderly patients, ethical objections against such a randomized controlled trial seem exaggerated. Furthermore, we had no information about failure of arteriovenous accesses before the successful first cannulation. However, this would probably result in an underestimation of the point estimates for the investigated risk factors. The general strength of this study was the large and well-defined Dutch cohort of incident hemodialysis patients with an arteriovenous access with available data on many patient characteristics, laboratory measurements, and death.

In conclusion, we showed that cardiovascular disease, prior catheter use, levels of albumin, hsCRP, and fetuin-A were associated with primary patency loss in both patients with a fistula and a graft. Current guidelines for prevention of vascular access failure recommend uniform surveillance of all patients.[33] The results of our study might lead to a more directed approach for surveillance techniques. The observed risk factors for primary patency loss could be used to focus on specific patient groups for more intensive surveillance. Furthermore, we showed that graft use as compared with fistula use was associated with an increased risk of primary patency and an increased mortality risk.

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Chapter 10

Single nucleotide variants in the protein C pathway and mortality in dialysis patients

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ABSTRACT

Background: The protein C pathway plays an important role in the maintenance of endothelial barrier function and in the inflammatory and coagulant processes that are characteristic of patients on dialysis. We investigated whether common single nucleotide variants (SNV) in genes encoding protein C pathway components were associated with all-cause 5 years mortality risk in dialysis patients.

Methods: Single nucleotides variants in the factor V gene (*F5* rs6025; factor V Leiden), the thrombomodulin gene (*THBD* rs1042580), the protein C gene (*PROC* rs1799808 and 1799809) and the endothelial protein C receptor gene (*PROCR* rs867186, rs2069951, and rs2069952) were genotyped in 1070 dialysis patients from the NEtherlands COoperative Study on the Adequacy of Dialysis (NECOSAD) cohort) and in 1243 dialysis patients from the German 4D cohort.

Results: Factor V Leiden was associated with a 1.5-fold (95% CI 1.1-1.9) increased 5-year all-cause mortality risk and carriers of the AG/GG genotypes of the PROC rs1799809 had a 1.2-fold (95% CI 1.0-1.4) increased 5-year all-cause mortality risk. The other SNVs in *THBD*, *PROC*, and *PROCR* were not associated with 5-years mortality.

Conclusion: Our study suggests that factor V Leiden and PROC rs1799809 contributes to an increased mortality risk in dialysis patients.

INTRODUCTION

The protein C pathway plays an important role in endothelial barrier function and in inflammatory and anticoagulant processes.[1] Protein C activation occurs on the endothelial cell membrane by thrombin bound to thrombomodulin and this is enhanced when protein C is bound to the endothelial protein C receptor. Activated protein C together with its cofactor protein S inactivates the procoagulant factors Va and VIIIa. Activated protein C resistance is often caused by a variant of factor V (factor V Leiden), that abrogates one of the inactivation sites in factor Va.[2] Besides anticoagulant properties, activated protein C has direct cytoprotective effects on endothelial cells that include anti-inflammatory actions, anti-apoptotic activities, and stabilization of endothelial barriers. These effects are largely mediated by activation of protease activated receptors.[3-7]

The crucial role of the protein C pathway in endothelial function, coagulation, and inflammation became evident in several studies.[8-12] The importance of the protein C system is most clearly demonstrated by the massive thrombotic complications occurring in infants with severe homozygous or compound heterozygous protein C deficiency[8] and the increased risk of venous thrombosis in haploinsufficient adults.[9] In severe sepsis patients with a high mortality risk treatment with activated protein C reduced mortality, probably through its anti-inflammatory and anticoagulant activities.[10] In addition, low plasma protein C levels have been shown to increase the risk of ischemic stroke.[11] Finally, particular combinations of variants in the thrombomodulin, protein C, and factor V genes seem to increase the risk of cardiovascular events in the general population.[12]

Patients on dialysis have a high mortality risk due to endothelial damage and subsequent cardiovascular diseases.[13] Dialysis patients also have a high risk of dying from dialysis treatment failure,[13] which is associated with thrombotic events (i.e. vascular access thrombosis and catheter thrombosis) and infections.[13] Genetic variation in the protein C pathway could influence the mortality risk by changing processes related to endothelial damage, by influencing inflammatory response, and by increasing or decreasing the chance of thrombotic events associated with treatment failure. We hypothesized that genetic mutations in genes encoding protein C pathway components or targets might influence mortality rates in dialysis patients.

We selected seven single nucleotide variants (SNVs) that are known to influence levels or activity of proteins in the protein C pathway or have been associated with venous thrombosis, arterial thrombosis or mortality in the general population: factor V (*F5)* rs6025 (factor V Leiden),[2] thrombomodulin (*THBD)* rs1042580,[12,14] protein C (*PROC)* rs1799809 and rs1799808,[15] and protein C receptor (*PROCR)* rs867186, rs2069952, and rs2069952.[16] We investigated the

association between these SNVs and all-cause and cause-specific (cardiovascular and non-cardiovascular) mortality in the NEtherlands COoperative Study on the Adequacy of Dialysis (NECOSAD) cohort and the German Diabetes Dialysis Study (4D-study).

METHODS

Patients

The Netherlands Cooperative Study on the Adequacy of Dialysis (NECOSAD) is a prospective multicenter cohort study in which incident adult dialysis patients from 38 dialysis centers in the Netherlands were included. The Medical Review Ethics Committee of the Leiden University Medical Center approved the study. All patients gave written informed consent. Eligibility criteria included age older than 18 years, and no previous renal replacement therapy (transplantation or dialysis). For the current analyses, we used data from patients who were included between June 1997 and June 2007 in 23 dialysis centers that approved DNA analysis. Information was gathered from patients until date of death or date of censoring, i.e. transfer to a nonparticipating dialysis center, withdrawal from the study, transplantation, or end of the follow-up period in June 2009, whichever occurred first.

Demographic and clinical data

Data on age, sex, primary kidney disease, and cardiovascular disease were collected at the start of dialysis treatment. Pre-existing cardiovascular disease was defined as a history of angina pectoris, myocardial infarction, heart failure, ischemic stroke, or claudication at the time of inclusion.

Single nucleotide variants

Blood samples were collected for DNA analysis. We genotyped one SNV in the factor V gene (*F5* rs6025; factor V Leiden), one SNV in the thrombomodulin gene (*THBD* rs1042580), two SNVs in the protein C gene (*PROC* rs1799809 and rs1799808), and three SNVs in the protein C receptor gene (*PROCR* rs867186, rs2069951, and rs2069952) using TaqMan SNV Genotyping Assays (Applied Biosystems, Foster City, CA, USA) as described previously.[14-17]

Mortality

We classified causes of death according to the codes of the European Renal Association-European Dialysis and Transplantation Association (ERA-EDTA) which is a standardized classification of death causes in dialysis patients.[18] We grouped death causes into cardiovascular and non-cardiovascular mortality. Cardiovascular mortality was defined as death due to myocardial ischemia and infarction (code 11); cardiac arrest/ sudden death (code 15); cardiac failure/ fluid overload/ pulmonary edema (codes 14,16,18); hyperkalemia /

hypokalemia (code 12,17); pulmonary embolism (code 21); cerebrum-vascular accident (code 22); hemorrhage from ruptured vascular aneurysm (code 26); mesenteric infarction (code 29); cause of death uncertain/unknown (code 0). All other deaths were designated as non-cardiovascular mortality.

Replication

For independent replication of the results of the NECOSAD study, we analyzed data from the German Diabetes Dialysis Study (4D-study). Methods of the 4D-study have been described in detail previously.[19] Briefly, the 4D-study was a double-blind, randomized trial on the effect of atorvastatin in hemodialysis patients with type 2 diabetes mellitus who had less than two years of previous hemodialysis treatment. The primary endpoint was a composite of cardiac death, non-fatal myocardial infarction and stroke, whichever occurred first. Patients were randomly assigned to either 20 mg of atorvastatin or placebo once daily until the date of death, censoring, or the end of study in March 2004. Atorvastatin showed no effect on the composite primary endpoint.[19] The genotyping of the SNV in the factor V gene (*F5* rs6025; factor V Leiden), the SNV in the thrombomodulin gene (*THBD* rs1042580), the two SNVs in the protein C gene (*PROC* rs1799809 and rs1799808), and the three SNVs in the protein C receptor gene (*PROCR* rs867186, rs2069951, and rs2069952) were the same as described above for the NECOSAD cohort. The SNV in the factor V gene (*F5* rs6025; factor V Leiden), the SNV in the thrombomodulin gene (*THBD* rs1042580), and the SNV in the protein C receptor gene (*PROCR* rs867186) were genotyped earlier than the other SNVs, therefore the numbers vary for these SNVs as compared with the other SNVs. Mortality was also categorized into cardiovascular and non-cardiovascular deaths.

Statistical analysis

The baseline characteristics are presented as median and 5^{th}-95^{th} percentiles for continuous variables, and as percentages for categorical variables. Distributions of genotypes were compared by the chi-square test to test for Hardy-Weinberg equilibrium. We calculated pooled hazard ratios (HRs) with 95% confidence intervals (95% CIs) for all-cause, cardiovascular, and non-cardiovascular mortality by Cox's regression analysis to study the effect of the seven SNVs on 5-year mortality from start of dialysis in the NECOSAD and 4D-study together. Furthermore, we calculated HRs with 95% CIs separately for the NECOSAD cohort and 4D-study. In addition, we repeated the analyses in the NECOSAD cohort for only hemodialysis patients with diabetes mellitus, since the 4D-study consists of only hemodialysis patients with diabetes mellitus. HRs were calculated for homozygous or heterozygous carriers of the rare alleles (except for rs2069952 for which the risk allele was the common major allele) of the SNVs compared to non-carriers. We reported unadjusted HRs, since adjustment in genetic association studies could potentially introduce interference in the causal pathway and thereby

bias through overadjustment.[20] We used SPSS statistical software (version 17.0; SPSS, Chicago) for all statistical analyses.

RESULTS

A total of 1070 patients from the NECOSAD cohort and 1243 patients from the 4D cohort were genotyped for the seven SNVs. Baseline characteristics of the 1070 patients from the NECOSAD cohort and 1243 patients from the 4D cohort are shown in Table 1. In contrast to the NECOSAD cohort, the 4D cohort consisted only of hemodialysis patients with diabetes mellitus. In the NECOSAD cohort, 140 hemodialysis patients (20.7%) had diabetes mellitus.

Table 1. Baseline characteristics

	NECOSAD N=1070	**4D-study N=1243**
Age (years), median (5^{th}-95^{th} percentile)	62.2 (33.0- 80.2)	66.0 (51.0-78.0)
Males, %	62.9	54.1
Body mass index (kg/m^2), median (5^{th}-95^{th} percentile)	24.3 (19.2-33.2)	26.7 (20.7-36.3)
Dialysis duration (months), median (5^{th}-95^{th} percentile)	0	6.0 (1.1-22.5)
Dialysis modality,%		
Hemodialysis	63.4	100
Peritoneal dialysis	36.6	0
History of diabetes mellitus, %	20.7	100
Cardiovascular disease,%	35.2	29.5

Table 2 shows the genotype and allele frequencies for the seven SNVs. All SNVs in the NECOSAD cohort were in Hardy-Weinberg equilibrium, except *PROC* rs1799809 (p-value 0.002). In the 4D cohort, *F5* rs6025 (factor V Leiden) (p-value <0.001), *PROC* rs1799808 (p-value <0.036), and *PROCR* rs2069952 (p-value 0.001) were not in Hardy-Weinberg equilibrium (Table 2).

Of the 1070 patients from the NECOSAD cohort, 401 died within 5 years of follow-up; 185 patients due to cardiovascular causes and 216 due to non-cardiovascular causes. In the 4D cohort, 594 patients died within 5 years of follow-up (297 patients due to cardiovascular causes and 297 due to non-cardiovascular causes).

Factor V (Leiden) rs6025

Factor V Leiden was associated with a 1.5-fold (95% CI 1.1-1.9) increased 5-year all-cause mortality risk in the pooled results. The hazard ratios were 1.4 (95% CI 0.9-2.1) in the total NECOSAD cohort and 1.6 (95% CI 1.1-2.2) in the 4D-study (Table 3). Restricting the analyses to diabetic patients with hemodialysis in the NECOSAD study (similar to the 4D study which

only includes diabetic hemodialysis patients), factor V Leiden was associated with a 2.1-fold (95% CI 1.0-4.5) increased 5-year all-cause mortality risk (Table 3).

Table 2. Distribution of single nucleotide variants

			NECOSAD N=1070			4D-STUDY N= 1243		
Gene	**SNV Location**	**Genotype**	**N**	**%**	**HW equilibrium**	**N**	**%**	**HW equilibrium**
Factor V (Leiden)	rs6025 exon	GG AG AA	984 53 2	(94.7) (5.1) (0.2)	*p*=0.157	837 48 4	(94.2) (5.4) (0.4)	*p*<0.001
Thrombomodulin	rs1042580 3'UTR	AA AG GG	404 479 157	(38.8) (46.1) (15.1)	*p*=0.443	321 437 132	(36.1) (49.1) (14.8)	*p*=0.397
Protein C	rs1799808 promoter	CC CT TT	431 478 140	(41.1) (45.6) (13.3)	*p*=0.681	491 604 143	(39.7) (48.8) (11.6)	*p*=0.036
Protein C	rs1799809 promoter	AA AG GG	368 461 215	(35.2) (44.3) (20.6)	*p*=0.002	402 620 215	(32.5) (50.1) (17.4)	*p*=0.363
Protein C receptor	rs867186 exon	AA AG GG	834 193 18	(79.8) (18.5) (1.7)	*p*=0.084	667 211 10	(75.1) (23.8) (1.1)	*p*= 0.136
Protein C receptor	rs2069951 intron	GG GA AA	919 130 6	(87.1) (12.3) (0.6)	*p*=0.549	1129 106 4	(91.1) (8.6) (0.3)	*p*= 0.372
Protein C receptor	rs2069952 intron	CC CT TT	164 493 383	(15.8) (47.4) (36.8)	*p*=0.798	263 552 424	(21.2) (44.6) (34.2)	*p*=0.001

Thrombomodulin, protein C and protein C receptor variants

As compared to the AA genotype in *PROC* rs1799809, carriers of the AG/GG genotypes had a 1.2-fold (95% CI 1.0-1.4) increased 5-year all-cause mortality risk (Table 3). *PROC* rs1799809, *THBD* rs1042580, *PROC* rs1799808, *PROCR* rs867186, *PROCR* rs2069951, and *PROCR* rs2069952 were not associated with all-cause mortality in the NECOSAD and the 4D cohorts (Table 3).

Table 3. Effect of single nucleotide variants on 5-year mortality

			POOLED RESULTS N=2313		NECOSAD N=1070		NECOSAD HD and DM* N=140		4D-STUDY N=1243	
Gene/SNV	**Genotype**	**Mortality**	**HR**	**(95% CI)**	**HR**	**(95% CI)**	**HR**	**(95% CI)**	**HR**	**(95% CI)**
Factor V (Leiden)/	GG				1	(reference)	1	(reference)	1	(reference)
rs6025	AG/AA	All-cause	1.5	(1.1-1.9)	1.4	(0.9-2.1)	2.1	(1.0-4.5)	1.6	(1.1-2.2)
		CV	1.5	(1.0-2.2)	1.3	(0.7-2.4)	0.5	(0.1-3.9)	1.6	(1.0-2.6)
		Non-CV	1.5	(1.0-2.2)	1.4	(0.8-2.5)	4.0	(1.7-9.5)	1.6	(0.9-2.6)
Thrombomodulin/	AA				1 1.0	(reference)	1	(reference)	1	(reference)
rs1042580	AG/GG	All-cause	1.0	(0.9-1.1)	0.9	(0.8-1.2)	1.2	(0.8-1.9)	1.0	(0.8-1.2)
		CV	1.0	(0.8-1.2)	1.0	(0.7-1.2)	1.0	(0.5-1.9)	1.0	(0.8-1.3)
		Non-CV	1.0	(0.8-1.2)		(0.8-1.4)	1.4	(0.7-2.8)	1.0	(0.8-1.3)
Protein C/	CC				1	(reference)	1	(reference)	1	(reference)
rs1799808	CT/TT	All-cause	1.0	(0.9-1.2)	1.1	(0.9-1.3)	1.1	(0.7-1.7)	1.0	(0.8-1.2)
		CV	1.0	(0.8-1.2)	0.9	(0.7-1.3)	0.8	(0.4-1.5)	1.0	(0.8-1.3)
		Non-CV	1.0	(0.9-1.2)	1.2	(0.9-1.5)	1.5	(0.8-3.0)	1.0	(0.8-1.2)
Protein C/	AA				1	(reference)	1	(reference)	1	(reference)
rs1799809	AG/GG	All-cause	1.2	(1.0-1.4)	1.2	(0.9-1.5)	0.9	(0.5-1.4)	1.2	(1.0-1.4)
		CV	1.2	(1.0-1.5)	1.2	(0.9-1.6)	1.0	(0.5-2.0)	1.3	(1.0-1.6)
		Non-CV	1.2	(1.0-1.4)	1.2	(0.9-1.6)	0.8	(0.4-1.5)	1.1	(0.9-1.5)
Protein C receptor/	AA				1	(reference)	1	(reference)	1	(reference)
rs867186	AG/GG	All-cause	1.0	(0.8-1.1)	0.9	(0.7-1.2)	1.3	(0.8-2.2)	1.0	(0.8-1.2)
		CV	0.8	(0.6-1.0)	0.7	(0.5-1.0)	1.0	(0.4-2.1)	0.8	(0.6-1.1)
		Non-CV	1.2	(1.0-1.5)	1.2	(0.9-1.6)	1.7	(0.9-3.4)	1.2	(0.9-1.5)
Protein C receptor/	GG				1	(reference)	1	(reference)	1	(reference)
rs2069951	AG/AA	All-cause	1.1	(0.9-1.4)	1.1	(0.8-1.5)	1.0	(0.5-1.9)	1.2	(0.9-1.6)
		CV	1.2	(0.9-1.5)	1.0	(0.7-1.6)	1.3	(0.5-3.1)	1.4	(0.9-1.9)
		Non-CV	1.1	(0.8-1.4)	1.2	(0.8-1.7)	0.6	(0.2-2.0)	1.0	(0.7-1.5)
Protein C receptor/	CC				1	(reference)	1	(reference)	1	(reference)
rs2069952	CT/TT	All-cause	1.0	(0.8-1.1)	0.9	(0.7-1.2)	2.3	(1.2-4.5)	1.1	(0.9-1.3)
		CV	1.0	(0.8-1.2)	0.9	(0.6-1.3)	1.5	(0.7-3.4)	1.1	(0.8-1.4)
		Non-CV	1.0	(0.8-1.2)	1.0	(0.7-1.4)	4.1	(1.3-13.4)	1.0	(0.8-1.4)

*hemodialysis patients with diabetes mellitus

DISCUSSION

This candidate-gene study assessed the 5-year mortality risk while on dialysis treatment for seven genetic variants that influence levels or activity of proteins in the protein C pathway: one SNV in the factor V gene (factor V Leiden), two SNVs in the protein C gene (*PROC*), one SNV in the thrombomodulin gene (*THBD*) and three SNVs (tagging three haplotypes) in the protein C receptor gene (*PROCR).* We found that factor V Leiden was associated with a 1.5-fold (95% CI 1.1-1.9) increased 5-year all-cause mortality risk and that *PROC* rs1799809 was associated with a 1.2-fold (95% CI 1.0-1.4) increased 5-year all-cause mortality risk. Furthermore, we showed that *THBD* rs1042580, *PROC* rs1799808, *PROCR* rs867186, *PROCR* rs2069951, and *PROCR* rs2069952 were not associated with an increased mortality risk.

Studies in the general population have shown an association between factor V Leiden and an increased risk of different adverse outcomes, including venous thrombosis,[17] ischemic stroke,[21] and myocardial infarction.[22] We showed in the current study that factor V Leiden was associated with increased all-cause mortality in dialysis patients. Other studies did not find an increased all-cause mortality risk for factor V Leiden in the general population and in thrombophilic families.[23,24] However, it could be that an interaction between dialysis and factor V Leiden leads to an increased mortality risk in dialysis patients. Previous studies on factor V in the dialysis population have been focused on arteriovenous access failure. A recent study showed that a factor V gene SNV (rs6019) was associated with arteriovenous graft failure in dialysis patients suggesting an association between factor V SNVs and adverse outcomes in dialysis patients,[25] which is in line with our study.

Several mechanisms might provide plausible explanations for the higher mortality risk in dialysis patients associated with factor V Leiden. First, factor V Leiden in combination with pre-existing and highly prevalent endothelial damage could lead to excess mortality from cardiovascular events. Second, factor V Leiden has been associated with venous thrombosis.[14,16,17,26] The excess mortality could have been caused by fatal pulmonary embolisms due to the procoagulant changes due to factor V Leiden in combination with the start of dialysis which is also associated with an increased risk of venous thrombosis.[27,28] Arguing against this explanation is that in our study confirmed pulmonary embolism was the cause of death in only three patients, but pulmonary embolisms as cause of death might have gone undetected or misclassified as for example sudden cardiac death. Third, one of the main complications in dialysis therapy is clot formation and thrombosis in vascular accesses.[29] Factor V Leiden is associated with procoagulant changes and could therefore lead to treatment failure in dialysis patients. Previous studies have reported an increased risk of arteriovenous access failure in patients with factor V SNVs.[30,31]

THBD rs1042580 AG/GG genotypes have been associated with venous thrombosis in the general population.[14] In addition, the combination of *THBD* rs1042580 with different Factor V SNVs was associated with an increased risk of cardiovascular events.[12] Other *THBD* SNVs have also been associated with cardiovascular outcomes when combined with *PROC* SNVs or factor V Leiden in the general population.[12] However, we did not find an association between *THBD* rs1042580 AG/GG genotypes and mortality in dialysis patients.

In contrast to previous studies in sepsis patients, we found no association between the *PROC* rs1799808 and mortality. However, we found that *PROC* rs1799809 was associated with a small increased (hazard ratio 1.2, 95% CI 1.0-1.4) mortality risk. Haplotypes tagged by these SNVs have been associated with decreased survival and increased organ dysfunction in

severe sepsis patients.[32,33] An earlier study found that *PROC* rs1799808 was less important in the determination of protein C levels, indicating that the effect on protein C levels was mainly mediated by the *PROC* rs1799809.[15] Studies in the general population on *PROCR* rs867186, *PROCR* rs2069951, and *PROCR* rs2069952 have been inconsistent in the risk of venous thrombosis[15,16,26] and arterial thrombosis.[34,35] We did not find an association between these SNVs and mortality.

The genotype distribution of *PROC* rs1799809 deviated from Hardy-Weinberg equilibrium in the NECOSAD study and the genotype distribution of *F5* rs6025 (factor V Leiden), *PROC* rs1799808, and *PROCR* rs2069952 were not in Hardy-Weinberg equilibrium in the 4D cohort. It is likely that in diseased populations, such as dialysis patients, selection could have resulted in deviations from Hardy-Weinberg equilibrium.

A potential limitation of our study is that we replicated our results in a dialysis population consisting of hemodialysis patients with diabetes mellitus. For most of the SNVs this was not a problem, since there were no large differences when we restricted the NECOSAD cohort to hemodialysis patients with diabetes mellitus. However, in the 4D-study, we could not investigate the association between mortality and the protein C SNVs in peritoneal dialysis patients and in patients without diabetes mellitus. Furthermore, although we included more than 2000 dialysis patients, our study could be underpowered to detect small differences.

In conclusion, our study suggests that factor V Leiden and *PROC* rs1799809 contributes to an increased mortality risk in dialysis patients. This study is the first to investigate the association between protein C pathway SNVs and mortality in large cohorts of dialysis patients.

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Chapter 11

Summary and general discussion

11. INTRODUCTION

The main objectives of this thesis were to:

- investigate the association between kidney disease and venous and arterial thrombosis
- provide insight in the mechanism of the association between kidney disease and thrombosis
- investigate the mortality risks for hemodialysis patients with catheter, fistula or graft vascular accesses
- investigate the association between genetic risk factors for arterial and venous thrombosis and mortality in dialysis patients

In this chapter, the main findings are summarized and strengths and limitations of our studies are discussed. In addition, clinical implications, recommendations for future research and main conclusions are provided.

11.1 Main findings

In **chapter 2**, we investigated the association between self-reported liver disease, kidney disease, rheumatoid arthritis, multiple sclerosis, heart failure, hemorrhagic stroke and arterial thrombosis in a large case-control study (MEGA study). We showed that self-reported kidney disease was associated with an almost 4-fold increased risk of venous thrombosis. Liver disease, rheumatoid arthritis, multiple sclerosis, heart failure, hemorrhagic stroke and arterial thrombosis were also associated with an increased risk of venous thrombosis. Furthermore, we showed that combinations of these major illnesses with immobilization, increased factor VIII levels, increased factor IX levels, increased von Willebrand factor levels, factor V Leiden, and blood group non-O further increased the risks of venous thrombosis.

In **chapter 3**, we investigated the association between the early stages of kidney disease and venous thrombosis in a large cohort including 8495 subjects (PREVEND study). Most individuals with early stages of kidney disease are asymptomatic and unaware of their decreased kidney function or the presence of albuminuria. The incidence rate of venous thrombosis for patients with chronic kidney disease stages 1–3 was 3.7 per 1000 person-years. Patients with chronic kidney disease stages 1–3 had an almost 2-fold increased risk of venous thrombosis as compared with subjects without chronic kidney disease.

As kidney disease appeared to be associated with venous thrombosis in **chapters 2 and 3**, we investigated in the MEGA study (**chapter 4)** whether the association between kidney disease and venous thrombosis could be explained by body mass index, immobilization, surgery, corticosteroid use, diabetes mellitus, malignancy, arterial disorders, factor V Leiden,

prothrombin G20210A, and coagulation factors levels. We showed that a moderately to severely decreased kidney function (estimated glomerular filtration rate <60 ml/min) was associated with an almost 3-fold increased risk of venous thrombosis as compared with normal kidney function. Furthermore, we found that factor VIII levels and von Willebrand factor levels were increased in patients with a moderately to severely decreased kidney function. Adjustment for factor VIII or von Willebrand factor in the association between decreased kidney function and venous thrombosis attenuated the risk of venous thrombosis indicating an effect of kidney function on thrombosis through these factors. In contrast, adjustments for body mass index, factor V Leiden, prothrombin G20210A, diabetes mellitus, malignancy, arterial disorders, immobilization, surgery or corticosteroid use did not affect the risks of venous thrombosis.

In chapter 2 and 3, kidney disease was associated with venous thrombosis. In **chapter 5,** we wanted to identify high-risk groups with kidney disease that may benefit from thromboprophylaxis. Therefore, we investigated joint effects of reduced kidney function and common risk factors for venous thrombosis in the MEGA study. Moderately and severely reduced kidney function in combination with arterial thrombosis resulted in a 5-fold increased risk of venous thrombosis, with malignancy in a 6-fold increased risk, with surgery in a 14-fold increased risk, with immobilization in a 17-fold increased risk, with the factor V Leiden mutation in a 4-fold increased risk and with the prothrombin G20210A mutation in a 10-fold increased risk of venous thrombosis. The risks of venous thrombosis increased further in the presence of more than one risk factor of venous thrombosis in combination with moderately and severely decreased kidney function.

Chapter 2 to 4 specifically focused on non-dialysis patients. In **chapter 6**, we assessed the absolute risk of venous thrombosis, myocardial infarction and ischemic stroke in a cohort of end-stage renal disease patients receiving dialysis treatment (NECOSAD). The incidence rate of venous thrombosis was 12 per 1000 person-years. The incidence rate of myocardial infarction was 62 per 1000 person-years. For ischemic stroke, we found an incidence rate of 28 per 1000 person-years. The incidence rates in dialysis patients as compared with the general population were 6-fold increased for venous thrombosis, 12-fold increased for myocardial infarction, and 8-fold increased for ischemic stroke after adjustment for age and sex.

In the previous chapter, we mainly investigated non-fatal cases of venous thrombosis, myocardial infarction and stroke. In **chapter 7**, we evaluated mortality rate ratios for pulmonary embolism, myocardial infarction and stroke in dialysis patients from the ERA-EDTA registry as compared with the general population. The age- and sex-standardized mortality rate from pulmonary embolism was 12 times higher in dialysis patients than in the general population.

For myocardial infarction and stroke, we showed that dialysis patients had, respectively, an 11-fold and 8-fold increased mortality risk as compared with the general population after adjustment for age and sex.

In **chapters 8 and 9**, we focused on vascular access related complications in hemodialysis patients. We found that the mortality rate was 1.5-fold higher in patients with a catheter for hemodialysis than in those with an arteriovenous access for hemodialysis. Especially elderly patients with a catheter as vascular access had high mortality rates on dialysis. Furthermore, we showed that graft use as compared with fistula use in hemodialysis patients with a vascular access was associated with a 1.4-fold increased risk of primary patency loss and with a 1.5-fold increased mortality risk.

In chapters 6 to 9, we showed that dialysis patients were at increased risk of vascular access related complications and venous and arterial thrombosis. In **chapter 10**, we investigated whether polymorphisms in the protein C pathway (factor V Leiden, *THBD* rs1042580, *PROC* rs1799808 and 1799809 and *PROCR* rs867186, rs2069951, and rs2069952) were associated with mortality in dialysis patients in the NECOSAD cohort and the German 4D cohort. The protein C pathway plays an important role in endothelial barrier function and anticoagulant processes and abnormalities in this pathway are associated with venous or arterial thrombosis or vascular access related complications. Factor V Leiden was associated with a 1.5-fold increased 5-year all-cause mortality risk and carriers of the AG/GG genotypes of the PROC rs1799809 had a 1.2-fold increased 5-year all-cause mortality risk in the pooled cohorts. The other genotyped single nucleotide variants in the thrombomodulin gene (*THBD* rs1042580), the protein C gene (*PROC* rs1799808 and 1799809), and the endothelial protein C receptor gene (*PROCR* rs867186, rs2069951, and rs2069952) were not associated with 5-year all-cause mortality.

11.2 Strengths and limitations

In this section, the strengths and limitations of our studies in the MEGA study, PREVEND study, NECOSAD study, 4D study and the ERA-EDTA registry are discussed.

11.2.1 MEGA study

The studies described in **chapter 2, 4 and 5** are based on data collected from the MEGA study. The MEGA study is a large, population-based case-control study on risk factors for venous thrombosis. The major strengths of the MEGA study include the large patient sample, the detailed information about established risk factors in both patients and controls, and the presence of blood samples for creatinine measurements.

A limitation of the MEGA study is that blood samples were collected after the thrombotic event. Therefore, we cannot exclude the possibility that differences in creatinine levels between cases and controls resulted from the thrombotic event itself. However, it is not likely that thrombotic events influence creatinine levels. Furthermore, we showed that there were no major differences in estimated glomerular filtration rates when patients were tested within 3-6 months, 6-12 months or after 12 months suggesting that creatinine levels were not influenced by a temporally raised effect.

Another limitation was that those who died soon after a first venous thrombotic event (4% of the patient population) could not participate as a case in the MEGA study. This has probably led to an underestimation of our risk estimates, as patients with chronic kidney disease are more likely to die from venous thrombosis than patients without a major illness.[1,2]

In addition, 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 the association between decreased kidney function and venous thrombosis can be explained by the presence of proteinuria. Proteinuria, especially in the nephrotic range (defined as proteinuria of more than 3 grams per 24 hours), has been associated with venous thrombosis, which may be caused by changes in the plasma levels of some proteins involved in coagulation.[3-6]

Moreover, 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, since the thrombosis risk could be elevated for only specific primary kidney diseases.

A final aspect of the MEGA study was the presence of two separate control groups (partner controls and random digital dialing). While both may serve a slightly different purpose, results pointed in the same direction and were roughly similar when both control groups were analyzed separately.

11.2.2 PREVEND study

Major strengths of the PREVEND study as compared with the MEGA study are the presence of data on albuminuria which was assessed in 24-h urine samples and the presence of follow-up data to calculate absolute risks. Therefore, in the PREVEND study, absolute risks of venous thrombosis could be calculated for chronic kidney disease stages 1 and 2, since both information on albuminuria and kidney function are needed for these stages.

An important limitation of this cohort study was that the kidney function and the presence of albuminuria were assessed long before the occurrence of the disease (mean 4.0 years), resulting in a possible dilution of the effect.

Another limitation was that venous thrombotic events were identified through anticoagulation clinic databases and registers for hospital discharge diagnoses and death certificates, which could lead to an underestimation of the incidence rates of venous thrombosis by missing patients who had venous thrombosis. However, the incidence rates for venous thrombosis in the PREVEND cohort (i.e. 1.4 per 1000 person-years) correspond well to those found in previous studies.[7]

Furthermore, there were only 52 subjects in the PREVEND study with an estimated glomerular filtration rate below 45 ml/min. Therefore, we had a limited power to investigate the association between venous thrombosis and an estimated glomerular filtration rate below 45 ml/min. Previous studies suggested that especially these patients had increased risks of adverse outcomes, such as cardiovascular diseases and mortality.[8,9]

11.2.3 NECOSAD study

The NECOSAD study is a large and well-defined Dutch cohort of incident hemodialysis patients with available data on many patient characteristics, laboratory measurements, and death.

Our studies in the NECOSAD cohort have some potential limitations that should be addressed. A limitation of our studies is that comparisons between types of vascular access (catheter, fistula or graft) in an observational design could be a problem due to confounding by indication. Differences in outcomes for different treatment modalities in dialysis patients may reflect the different prognosis at baseline. In our analyses, we took this into account by correcting for these confounders, but this cannot exclude possible residual confounding.

Another limitation of this study is that there were missing values for several laboratory values, such as the kidney function and serum albumin. Analyses excluding patients who had a missing values could lead to biased results in case missing is influenced by the treatment or outcome.[10] Therefore, imputation could lead to more reliable results than excluding the patients with missing data.[10,11]

Moreover, we had no detailed information about the type of vascular access. Data about the type of catheters (tunnelled or non-tunnelled), the insertion place of the catheters and the use of antimicrobial locks for catheters were not present. We also had no information on

several arteriovenous access characteristics (anatomic location, flow, vessel diameter, and intervention prior to cannulation) that are associated with vascular access dysfunction.

Furthermore, we had no information about failure of arteriovenous access before the first dialysis session. Failure of vascular access before successful cannulation for dialysis is higher for fistulas than for grafts.[12] Therefore, it could be that graft use as compared with fistula use could be beneficial in specific subgroups in terms of morbidity, including number of hospitalizations, and quality of life when we take into account the time period between creation of a vascular access and the first dialysis session.

11.2.4 4D-study

We investigated the association between seven single nucleotide variants and all-cause and cause-specific mortality in the NECOSAD cohort and replicated these findings in the German Diabetes Dialysis Study.

A potential limitation of this study is that we replicated our results in a dialysis population consisting of hemodialysis patients with diabetes mellitus. The NECOSAD cohort also includes non-diabetic patients and patients treated with peritoneal dialysis. Therefore, in the 4D-study, we could not investigate the association between mortality and singe nucleotide variants in peritoneal dialysis patients and in patients without diabetes mellitus.

11.2.5 ERA-EDTA Registry

The study cohort consisted of more than 100 000 incident dialysis patients derived from the European Renal Association-European Dialysis and Transplant Association (ERA-EDTA) Registry.[13] An important strength of this study, besides its large size, is the presence of data on renal replacement therapy, including date of birth, sex, primary kidney disease, date of start of renal replacement therapy, dialysis modality at baseline and during follow-up, and date and cause of death.

A potential limitation of our study was that the cause of death was unknown in approximately 14% of the dialysis patients compared with 2.0% of the general population. This difference can be explained by the different method for assigning the cause of death in dialysis patients as compared with the general population. Causes of death among patients on dialysis were recorded by the primary nephrologist. When a patient died at home or elsewhere outside the hospital, the nephrologist will have been dependent on information from others, and may more likely report a cause of death as unknown. Conversely, causes of death within the general population are, according to law, recorded by the physician who confirmed the death and thereafter sent the data to the statistics office, resulting in few missing causes of death.

11.3 Clinical implications and future research

Vascular complications such as venous and arterial thrombosis and vascular access (fistula, graft or catheter) related complications are associated with many hospitalizations and deaths in chronic kidney disease patients, especially in dialysis patients.[14-20]

In **chapter 2**, we showed that self-reported kidney disease was associated with a 3.7-fold increased risk of venous thrombosis. Furthermore, we showed that self-reported rheumatoid arthritis, multiple sclerosis, heart failure, hemorrhagic stroke and arterial thrombosis in these patients were associated with an increased risk of venous thrombosis varying from a 1.5-fold increased risk for a history of arterial thrombosis and rheumatoid arthritis to a 4.9-fold increased risk for hemorrhagic stroke. Also other studies found an association between venous thrombosis and including liver disease,[21,22] kidney disease,[5,6,23] rheumatoid arthritis,[21,24] multiple sclerosis,[25] heart failure,[21,26,27] hemorrhagic stroke,[28] and arterial thrombosis.[21,29,30] Based on these estimates, thromboprophylaxis in patients with a major illness seems unjustified, since the number needed to treat would be excessively high, while introducing a considerable risk of major bleeding. However, risks increased in the presence of immobilization or trombophilia.

In **chapter 3**, we showed that patients with chronic kidney disease stage 1 and 2, and patients with chronic kidney disease stage 3 in the presence of albuminuria had increased risks of venous thrombosis. Patients with chronic kidney disease stage 3 without albuminuria had no increased risk of venous thrombosis. These findings are in line with several other studies suggesting a higher risk for chronic kidney disease stage 3 subjects with albuminuria than for CKD stage 3 subjects without albuminuria for different adverse outcomes, such as cardiovascular disease and the development of end-stage renal disease.[9,31-33] Based on the weak associations between early stages of chronic kidney disease and venous thrombotic risk (the incidence rate of subjects without chronic kidney disease was 1.3 per 1000 person-years and the incidence rate of subjects with chronic kidney disease stages 1-3 was 3.7 per 1000 person-years), the number needed to treat (approximately 400) will be too high to justify thromboprophylaxis for all patients with chronic kidney disease stages 1-3. Further studies are needed to show whether venous thrombosis prophylaxis in subgroups of patients with early stages of chronic kidney disease in the presence of albuminuria will be safe and cost-effective, especially as the high risk of anticoagulant treatment-related major bleeding episodes applies to chronic kidney disease stages 4 and 5, and not to the early stages of chronic kidney disease.[34]

In **chapter 4**, it was found that impaired kidney function affected venous thrombosis risk via concurrently raised factor VIII and von Willebrand factor levels. An increased body mass index,[35,36] factor V Leiden,[37,38] prothrombin G20210A,[37,38] diabetes mellitus,[35,39] malignancy,[38,40]

arterial thrombosis,[41,42] immobilization,[38] surgery,[38] and corticosteroid use[43] could not explain the association between an impaired kidney function and venous thrombosis. 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,[44] 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 exact mechanism could be of clinical importance, since targeting the actual risk factor could also influence the risk of venous thrombosis. Targeting epiphenomena would not influence the risk of venous thrombosis.

In **chapter 5**, kidney function showed an inverse association with venous thrombosis risk with a nearly 6-fold increased risk for those with severely decreased kidney function (estimated glomerular filtration rate <30 ml/min). Those with additional risk factors had an even higher risk of thrombosis, particularly patients who were immobilized or underwent surgery (around 15-fold increased risk). Furthermore, there was a cumulative effect when several risk factors were present simultaneously with renal function impairment, with over 50-fold increased risks. Furthermore, we showed that a high glomerular filtration rate of more than 125 ml/min was also associated with an increased risk of venous thrombosis (odds ratio 1.4; 95% CI 1.0-1.9). A high glomerular filtration rate has been shown to be an indicator for early kidney disease and a predictor of cardiovascular disease.[45-48] Based on the odds ratios of venous thrombosis for decreased kidney function ranging from 1.1 for mildly decreased kidney function (estimated glomerular filtration rate 60-90 ml/min) to 5.5 for severely decreased kidney function (estimated glomerular filtration rate <30 ml/min), thromboprophylaxis is probably not justified in all patients with decreased kidney function since it does not seem to outweigh the increased bleeding risk associated with decreased kidney function.[49,50] Randomized clinical trials are needed to investigate whether prophylaxis with anticoagulant medication is beneficial for specific subgroups of patients with chronic kidney disease.

In **chapter 6 and 7**, it was found that dialysis patients have an increased risk for non-fatal and fatal myocardial infarction, stroke and venous thrombosis.[51,52] This finding was in contrast to autopsy studies that showed that venous thrombosis was less common in dialysis patients than in non-dialysis patients.[53-56] However, autopsy studies are likely to be biased for this kind of comparisons. Clinicians should be aware of the increased risk of this disorder in dialysis patients, since it is the most common preventable cause of hospital death.[57]

In **chapter 8 and 9**, we showed that catheter use compared with arteriovenous access use was associated with increased mortality. Furthermore, we showed that graft use as compared with fistula use was associated with an increased risk of primary patency loss and with an increased mortality risk. Our findings are consistent with the National Kidney Foundation Kidney Disease Outcome Quality Initiative guidelines[58] and the European Best Practice Guidelines[59] which recommend the use of a fistulas instead of grafts or catheters for vascular access. However, it could be that for subgroups grafts are good alternatives as first option for a vascular access, especially when we take into account that failure of vascular access before successful cannulation for dialysis is higher for fistulas than for grafts which we did not investigate in our studies.[12]

In chapter 10, our study showed that factor V Leiden was associated with an increased mortality risk in dialysis patients. Further studies are needed to explore the role of coagulation abnormalities in dialysis patients and to investigate the pathologic mechanisms of coagulation abnormalities in dialysis patients that leads to adverse outcomes. Recent studies also showed that single nucleotide variants in the factor V gene were associated with arteriovenous graft failure in dialysis patients.[60,61] However, the association between factor V Leiden and adverse outcomes in dialysis patients are weak and the prevalence of factor V Leiden is too low to decide on a strategy to screen all dialysis patients for factor V Leiden. Furthermore, it is unknown what the therapeutic consequence should be in case factor V Leiden is found in a dialysis patient.

11.4 Conclusions

The main conclusions of this thesis are:

- Kidney disease, rheumatoid arthritis, multiple sclerosis, heart failure, hemorrhagic stroke and arterial thrombosis are associated with an increased risk of venous thrombosis.
- Patients with chronic kidney disease stages 1–3 had an almost 2-fold increased risk of venous thrombosis as compared with subjects without chronic kidney disease.
- Impaired kidney function affects venous thrombosis risk via concurrently raised factor VIII and von Willebrand factor levels.
- Kidney function is inversely associated with venous thrombosis risk with a nearly 6-fold increased risk for those with severely decreased kidney function (estimated glomerular filtration rate <30 ml/min).
- Dialysis patients have an increased risk of fatal and non-fatal venous thrombosis, myocardial infarction and ischemic stroke
- Catheter use as compared with arteriovenous access use is associated with an increased mortality risk.

- Graft use as compared with fistula use is associated with an increased risk of primary patency loss and with an increased mortality risk.
- Factor V Leiden is associated with an increased mortality risk in dialysis patients.

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Nederlandse samenvatting

Dankwoord

Publicatielijst

Curriculum vitae

NEDERLANDSE SAMENVATTING

Vasculaire complicaties in nierziekte

Het aantal patiënten met een chronische nierziekte is de afgelopen jaren sterk toegenomen. Chronische nierziekte wordt vastgesteld op grond van het niveau van nierfunctie en nierschade. Het aantal volwassenen met een chronische nierinsufficiëntie in de Nederlandse bevolking wordt geschat op 10%. Chronische nierinsufficiëntie wordt in vijf stadia ingedeeld op basis van de nierfunctie en de aanwezigheid van nierschade (albuminurie). Individuen met stadium 5 chronische nierinsufficiëntie komen in aanmerking voor dialyse of transplantatie. Dialysepatiënten die dialyseren door middel van hemodialyse hebben een vaattoegang in de vorm van een fistel (de verbinding tussen ader en slagader zonder een kunststof prothese), een graft (de verbinding tussen ader en slagader met een kunststof prothese) of een catheter (kunststof slang die in een grote ader in de lies, de hals of onder het sleutelbeen wordt ingebracht).

Een groot gezondheidsprobleem bij patiënten met een nierziekte zijn stolselvormingen in slagaders (arteriële trombose), aders (veneuze trombose) en vaatproblemen gerelateerd aan vaattoegang bij patiënten op hemodialyse. Deze verschillende vaatproblemen leiden tot veel opnamen in het ziekenhuis, tot blijvende gezondheidsschade, tot een verminderde kwaliteit van leven en tot een hogere sterfte bij patiënten met een nierziekte. Het doel van het onderzoek beschreven in dit proefschrift was meer inzicht te krijgen in de relatie tussen nierziekte en trombose en om de mechanismen die leiden tot trombosevorming in patiënten met nierziekte te achterhalen. Verder werd de relatie onderzocht tussen bepaalde genetische varianten die een rol spelen in het ontwikkelen van veneuze en arteriële trombosen en sterfte van dialysepatiënten. Daarnaast werden de sterfterisico's voor dialysepatiënten met een fistel, graft en catheter bestudeerd.

In **hoofdstuk 2** werd het risico op veneuze trombose voor zelfgerapporteerde nierziekten onderzocht in de MEGA studie (Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis). Patiënten met een nierziekte bleken een 4 keer hoger risico op veneuze trombose te hebben dan personen zonder een nierziekte.

Hoofdstuk 3 beschrijft de resultaten van een onderzoek naar de vroege stadia (stadium 1, 2 en 3) van nierziekte en de relatie met veneuze trombose in de PREVEND studie (Prevention of Renal and Vascular Disease). Er werd aangetoond dat patiënten met een nierziekte in stadium 1,2 en 3 een bijna 2 keer verhoogd risico hadden op veneuze trombose.

Hoofdstuk 4 doet verslag van een onderzoek in de MEGA studie waarin werd onderzocht of de relatie tussen nierziekte en veneuze trombose verklaard kon worden door stollingsfactoren, body mass index, factor V Leiden, prothrombin G20210A, diabetes mellitus, maligniteit, arteriële trombose, immobilisatie of corticosteroid gebruik. We vonden dat verhoogde factor VIII en Von Willebrand factorwaarden de relatie tussen nierziekten en veneuze trombosen verklaarden.

In **hoofdstuk 5** onderzochten we de associatie tussen veneuze trombose en de combinatie van nierziekte met andere risicofactoren voor veneuze trombose in de MEGA studie om hoog-risico groepen te identificeren die baat zouden kunnen hebben van tromboseprofylaxe. Het bleek dat vooral patiënten met een nierziekte die geopereerd werden of immobiel waren een sterk verhoogd risico hadden op veneuze trombosen.

Hoofdstuk 6 rapporteert over een onderzoek waarin de risico's op veneuze trombose, myocardinfarct en herseninfarct werden bestudeerd voor dialysepatiënten in de NECOSAD studie (Netherlands Cooperative Study on the Adequacy of Dialysis). We toonden aan dat dialysepatiënten een 6 keer verhoogd risico hadden op veneuze trombose, een 12 keer verhoogd risico op een myocardinfarct en een 8 keer verhoogd risico op een herseninfarct.

In **hoofdstuk 7** werden de overlijdensrisico's op veneuze trombose, myocardinfarct en beroerte (herseninfarct of hersenbloeding) onderzocht voor dialysepatiënten in de ERA-EDTA database (European Renal Association-European Dialysis and Transplant Association). Het bleek dat dialysepatiënten een 12 keer verhoogd risico hadden op overlijden door een veneuze trombose, een 11 keer verhoogd risico op overlijden door myocardinfarct en een 8 keer verhoogd risico op overlijden door een beroerte in vergelijking met de algemene bevolking.

Hoofdstuk 8 en 9 beschrijft de resultaten van een onderzoek in de NECOSAD studie waarin vaattoegangsproblemen werden onderzocht in dialysepatiënten. Het bleek dat patiënten met een catheter een hogere sterftekans hadden dan patiënten met een fistel of graft. Daarnaast toonden we aan dat patiënten met een graft in vergelijking met patiënten met een fistel een hogere kans hadden op het niet functioneren van de vaattoegang en een hogere kans op overlijden.

In **hoofdstuk 10** onderzochten we de relatie tussen genetische varianten in de 'Protein C pathway' (factor V Leiden, *THBD* rs1042580, *PROC* rs1799808 and 1799809 and *PROCR* rs867186, rs2069951, and rs2069952) en overlijden in dialysepatiënten in de NECOSAD studie en de Duitse 4D studie (German Diabetes Dialysis). De 'protein C pathway' speelt

een belangrijke rol in stollingsprocessen en in de vaten. Genetische varianten hierin zijn geassocieerd met arteriële en veneuze trombose. In deze studie bleek dat factor V Leiden was gerelateerd met een verhoogd overlijdensrisico in dialysepatiënten. De andere onderzochte genetische varianten waren niet geassocieerd met een verhoogd overlijdensrisico in dialysepatiënten.

In de verschillende studies in dit proefschrift kwam naar voren dat patiënten met een nierziekte een hoge kans hebben op veneuze trombose, arteriële trombose en op vaattoegangsproblemen. Deze bevindingen zouden tot klinische consequenties kunnen leiden. Gebaseerd op onze studies die aantoonden dat patiënten met een nierziekte een hoger risico hebben op veneuze trombose, zouden bepaalde patiënten met een nierziekte baat kunnen hebben van antistollingsmiddelen. Echter, de huidige antistollingsmiddelen geven een dermate hoog risico op ernstige bijwerkingen (bloedingen) dat tromboseprofylaxe in de vorm van anticoagulantia in alle patiënten met chronische nierziekte waarschijnlijk geen netto klinisch voordeel met zich zal meebrengen. Verder toonden we aan dat vooral dialysepatiënten met een catheter nadelige uitkomsten ondervinden in vergelijking met patiënten met een fistel of graft. Dit zou kunnen suggereren dat een catheter vermeden moet worden als vaattoegang. Grote, gecontroleerde, gerandomiseerde studies ontbreken echter. Verder onderzoek naar vasculaire complicaties in patiënten met een nierziekte is noodzakelijk voor het optimaliseren van de preventie en de behandeling van vasculaire complicaties in patiënten met een nierziekte.

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CURRICULUM VITAE

Gürbey Ocak is geboren op 6 november 1983 te Leiden in Nederland. Zijn middelbare school werd doorlopen aan het Visser 't Hooft college in Leiden waar hij in 2002 zijn gymnasium diploma behaalde (*cum laude*). In het zelfde jaar is hij begonnen aan zijn studie geneeskunde aan de Universiteit van Leiden waar hij in 2006 doctoraalexamen behaalde (*cum laude*) en in 2008 zijn artsendiploma behaalde (*cum laude*). Naast zijn studie heeft hij verscheidene bijbanen gehad onder andere als verzorger in een verpleegtehuis en als paramedische passagiersassistent op Schiphol. Al vroeg tijdens zijn studie deed hij ervaring op in het doen van wetenschappelijk onderzoek op de afdeling traumachirurgie naar prehospitale identificatie van polytraumapatiënten bij Prof. Dr. G.N. Jukema in het Leids Universitair Medisch Centrum, wat resulteerde in een publicatie. Tijdens zijn studie is hij geïnteresseerd geraakt in de nefrologie en epidemiologie en heeft hij zijn semi-arts stage op de afdeling Nierziekten/ Niertransplantatie gedaan en heeft hij een stage op de afdeling Klinische Epidemiologie gedaan. Na zijn artsexamen is hij in 2009 begonnen zijn promotieonderzoek naar vasculaire complicaties bij nierziekten patiënten op de afdeling Klinische Epidemiologie onder supervisie van Prof. Dr. F.R. Rosendaal en Prof. Dr. F.W. Dekker. Vanaf september 2012 is hij in opleiding tot internist onder leiding van Dr. A.B.M. Geers en Dr. W.J.W. Bos in het Antonius Ziekenhuis in Nieuwegein.