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C-reactive protein as predictor of death in end-stage diabetic nephropathy: Role of peripheral arterial disease

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C-reactive protein as predictor of death in end-stage diabetic nephropathy: Role of peripheral arterial disease.

Background. Patients with diabetes type 2 receiving dialysis therapy have a poor survival prognosis, mainly due to cardiovascular events. Increased C-reactive protein (CRP) levels, important in atherosclerosis, are associated with an increased risk for cardiovascular events. However, to date no study has shown the predictive value of CRP in relation to peripheral arterial disease stage.

Methods. We enrolled all 445 prevalent patients with diabetic nephropathy receiving maintenance hemodialysis in 30 centers in Southern Germany from August 1999 to January 2000 for prospective study until December 2003. At inclusion, CRP and a complete clinical phenotype, including peripheral arterial disease Fontaine Stage were determined. The primary end point was all-cause mortality.

Results. A total of 305 (68.5%) patients died. An increased log CRP at study inclusion was significantly associated with an increase in hazard ratio (HR) by multivariate Cox regression for all-cause (HR = 1.5, $P = 0.002$) and cardiac death (HR = 1.76, $P = 0.02$) in the entire collective. This result was applicable only to patients with peripheral arterial disease Fontaine stage IV ($N = 190$, multivariate HR = 1.75 for all-cause mortality, $P = 0.006$). Possibly due to inadequate power, we observed only an insignificant trend for CRP as predictor of all-cause death in patients without peripheral arterial disease or with less severe forms of peripheral arterial disease (HR = 1.36, $P = 0.08$).

Conclusion. In contrast to patients with peripheral arterial disease stage IV, patients with less severe atherosclerosis and elevated CRP are, if any, at less risk for cardiovascular mortality, possibly due to the difference in extent of affected vasculature and thus activated platelets and coagulation. Before judging the predictive value of CRP for mortality, peripheral vessel status should be determined.

Key words: dialysis, diabetic nephropathy, inflammation, survival, peripheral arterial disease, diabetes mellitus.

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Patients receiving maintenance hemodialysis for end-stage renal disease (ESRD) have a dismal survival and morbidity prognosis [1, 2]. Cardiovascular events account for almost 50% of deaths [3]. Peripheral arterial disease has a prevalence of approximately 20% in incident dialysis patients [4, 5], with 17% of patients having a history of critical limb ischemia or knee amputation [5].

Local vascular inflammation, probably increased in diabetes mellitus due to the hyperglycemic state and oxidative stress [6], is an important factor in the pathogenesis of atherosclerosis [7]. C-reactive protein (CRP) may be a valuable marker of this process [8, 9], while there is also evidence for a causal role of CRP in atherosclerosis [10, 11]. There are ample data showing that increased CRP is predictive for cardiovascular end points in healthy individuals [8, 9] and dialysis patients [12–20], but CRP has been shown not to correlate well with the extent of vessel surface affected by atherosclerosis [21]. It has not been shown whether mortality risk predicted by CRP [8, 9, 12–20] is due to the extent of underlying atherosclerotic morbidity, inflammation per se or a combination of both. Recently, the importance of CRP relative to traditional risk factors in predicting cardiovascular end points has been questioned [9, 22, 23]. Remarkably, to date, there is no data showing the predictive value of CRP levels in patients in relation to peripheral artery disease stage, an important risk factor possibly causing population stratification [24]. Thus, we examined the role of CRP in predicting all-cause and cardiovascular mortality in a large cohort of well-classified dialysis patients with type 2 diabetes mellitus in a prospective multicenter study.

METHODS

Subjects

A total of 445 Caucasian patients with type 2 diabetes mellitus and ESRD were recruited from 30 dialysis centers in Southern Germany from August 1999 to January 2000. All prevalent patients of each dialysis center with

Table 1. Clinical characteristics of patients with and without peripheral arterial disease (PAD) stage IV

PAD stage IV	No PAD, PAD stages I-III ($N = 255$)	PAD stage IV ($N = 190$)	P value
Male gender %	51.0	60.0	0.045
Age at inclusion *years*	67.6 ± 8.6	67.3 ± 7.7	0.65
Duration hemodialysis at inclusion *years*	2.3 ± 1.9	2.8 ± 2.4	0.016
Diabetes duration at start of dialysis *years*	14.3 ± 9.6	16.7 ± 9.4	0.01
Body mass index	27.0 ± 4.8	26.1 ± 4.0	0.04
Hemoglobin A_{1c} %	6.94 ± 1.0	6.8 ± 1.3	0.3
C-reactive protein *mg/L*	11.5 ± 13.7	15.6 ± 17.8	0.005
Serum albumin *g/L*	42.5 ± 5.5	42.6 ± 4.8	0.5
present- or ex-smoker %	39.3	50.8	0.045
Coronary artery disease %	51.4	65.3	0.004
Myocardial infarction %	27.3	29.5	0.625
Coronary intervention %	16.7	20.0	0.38
Cerebral ischemia %	31.0	31.1	0.99
Angiotensin-converting enzyme inhibitor %	53.7	52.4	0.78
Angtiotensin II type 1 receptor inhibitor %	15.3	13.8	0.65
Platelet inhibitor %	50.2	62.4	0.01
HMG-CoA inhibitor %	27.8	26.8	0.82
Calcium channel blocker %	47.8	45.5	0.625
Beta blocker%	29.4	20.6	0.036
Dialysis time per week *hours*	12.5 ± 1.2	12.8 ± 1.3	0.1
High-flux dialysis filter %	47.6	39.5	0.2
Biocompatible membrane %	77.9	69.9	0.13
Survival from inclusion *years*	2.67 ± 1.38	2.24 ± 1.39	0.001

HMG-CoA is 3-hydroxy-3-methylglutaryl coenzymeA. Statistical testing was performed with χ^2 or two-sided t test, where applicable.

diabetic nephropathy due to diabetes mellitus type 2 were included. Diagnosis of diabetic nephropathy was either confirmed by biopsy, or assumed if there was a typical clinical course of longstanding diabetes mellitus successively followed by microalbuminuria, proteinuria, and renal insufficiency, in the absence of other causes of proteinuria. Patients were recruited only if age was >35 years at diagnosis of diabetes mellitus. Patients with clinical signs of systemic or overt local infection were excluded ($N = 13$). Clinical characteristics of the patients are displayed in Tables 1 and 2. The study was approved by the Ethics Committee of the Medical Faculty of the University of Regensburg [study number 97/38, "Genetic and Clinical Predictors of Morbidity, Mortality and Diabetic Nephropathy With End-Stage Renal Disease in Diabetes Mellitus Type 2—A Prospective Cohort Study" (GENDIAN)]. All patients gave informed consent to participation in the study.

Clinical parameters

At study inclusion, we determined cardiovascular risk profile and morbidity, medication history, laboratory parameters relevant to cardiovascular diseases, dialysis filter type, and weekly duration of dialysis by questionnaire and reviewing the patients' charts. We determined date of birth and of diagnosis of diabetes mellitus, nephropathy, and beginning of dialysis therapy, respectively. Specifically, date of onset of diabetes mellitus was determined as the date when a test for blood glucose was first abnormally high (either fasting blood glucose or glucose tolerance testing) and when the patient first took antidiabetic drugs. In addition, the patient chart was reviewed to obtain the date of diabetes onset. Peripheral arterial disease status was classified clinically according to the Fontaine classification. Hereby, patients with angiographically proven, asymptomatic atherosclerotic lesions of lower extremity vessels were classified as Fontaine stage I, those with intermittent claudication as stage II, and patients with resting claudication as stage III. Patients with extremity necrosis or amputation due to atherosclerotic vascular disease were classified as Fontaine stage IV. Care was taken not to misclassify patients with neuropathic foot lesions as Fontaine stage IV. Patients were followed until December 4, 2003. Primary end point was all-cause mortality. Cause of death was assessed where possible. Death due to myocardial infarction, cerebral ischemia, malignant arrhythmia, intracerebral hemorrhage, or acute cardiac failure was defined as the combined secondary end point, "cardio- and cerebrovascular death" death due to myocardial infarction, malignant arrhythmia, or acute cardiac failure was defined as the combined secondary end point, "cardiac death," death, due to pneumonia or septicemia of any cause as "infectious death" and death due to trauma, gastrointestinal bleeding, cancer, or liver cirrhosis was categorized as "other."

Specimen collection

Ten milliliters whole blood samples were drawn prior to hemodialysis sessions, and centrifuged within 6 hours. Serum was frozen at −80°C until analyses were performed.

Serum parameters

CRP was determined with the C-Reactive Protein Assay (Biomed, Oberschleissheim, Germany) (product number 123440). The assay was calibrated for a detection range of 0.1 to 320 mg/L according to the instructions of the producer. The intra-assay coefficients of variation were 1.2%, 0.79%, and 0.91% for the CRP concentrations 2, 8, and 24 mg/L, respectively. The interassay coefficient of variation was <2% for all concentrations.

Statistical analysis

Results are expressed as mean (±1 SD), unless stated otherwise. CRP values were grouped in quartiles. Comparisons of continuous variables between groups were performed by Student t test, analysis of variance (ANOVA), or by Kruskal-Wallis tests and of categoric variables by χ^2 or Fisher's exact test where applicable.

Table 2. Morbidity and causes of death of study collective

	C-reactive protein quartiles *mg/L*				
	All patients ($N = 445$)	1 (<3.7) ($N = 111$)	2 (3.7 to 7.5) ($N = 111$)	3 (7.51 to 17.35) ($N = 112$)	4 (17.36 to 96.7) ($N = 111$)
Coronary artery disease%	57.5	51.9	56.9	56.3	65.1
Myocardial infarction%	28.3	30.6	25.7	25.9	31.1
Coronary intervention%	18.2	16.7	19.3	20.5	16.0
Cerebral ischemia%	31.0	29.6	28.4	31.3	34.9
Peripheral arterial disease%	67.4	64.0	65.8	68.8	66.7
Fontaine stage I%	1.1	0	1.8	1.8	0.9
Fontaine stage II%	20.5	25.1	23.4	24.1	9.0
Fontaine stage III%	3.1	1.8	4.5	1.8	4.5
Fontaine stage IV%	42.7	36.9	37.8	42.9	53.2
Cardiovascular and cerebrovascular death%	118 (26.4)	19.8	27.5	24.1	34.2
Death due to infection%	55 (12.4)	8.1	13.8	15.2	12.6
Other cause of death%	19 (4.3)	5.4	3.7	5.4	2.7
Death cause unknown%	113 (23.7)	25.2	19.3	25.9	24.3

There are no differences in variables between C-reactive protein quartiles (χ^2 analysis, $P > 0.05$).

Statistical significance in all tests was accepted at $P < 0.05$.

Survival analysis was performed by the Kaplan-Meier method, comparing groups using the log-rank test. Censoring is performed for renal transplantation ($N = 9$ in non-peripheral arterial disease stage IV group and $N = 2$ in peripheral arterial disease stage IV group), loss to follow-up ($N = 2$ in peripheral arterial disease stage IV group) and if the patient is alive at the last examination ($N = 41$ in peripheral arterial disease stage IV group, $N = 86$ in non-peripheral arterial disease stage IV group). Duration of dialysis therapy from study inclusion onwards was the time variable if not indicated otherwise. Covariates used for survival analysis and explorative statistics for comparison of patient groups were log CRP (log of CRP value measured in mg/L), age at start of dialysis therapy (years), duration of previous dialysis therapy and of diabetes at study inclusion (years), gender (reference: female), smoking history (reference: never-smoker), body mass index, systolic-diastolic blood pressure difference prior to the dialysis session at inclusion (mm Hg), serum albumin (g/L), medication with angiotensin-converting enzyme (ACE) or angtiotensin II (Ang II) receptor 1 antagonists, 3-hydroxy-3-methylglutaryl coenzymeA (HMG-CoA) reductase inhibitors, platelet inhibitors (acetylsalicylic acid, ticlopidin, or clopidogrel) calcium channel antagonists and beta blockers (reference: no therapy), presence of coronary artery disease (reference: no coronary artery disease), history of myocardial infarction and cerebral ischemia (reference: no such history) and history of coronary intervention including bypass surgery (reference: no intervention).

To correct for covariates, a Cox proportional hazard ratio (HR) model was applied. First, the univariate HR was determined for each variable in each subgroup (patients with peripheral arterial disease stage IV vs. patients without peripheral arterial disease or with peripheral arterial disease stages I to III). Different Cox regression models were then performed with varying degrees of covariate adjustment. In model 1, effect of log CRP was adjusted for age at start of dialysis and previous duration of dialysis, in model 2 for age at start of dialysis therapy, previous duration of dialysis therapy, gender, smoking history and body mass index. In model 3, medication history was added in addition to the variables of model 2. In the extended model 4, all covariates were included. The fully adjusted model 4 was used to examine the effect of log CRP on secondary end points.

We found significant statistical interaction of peripheral arterial disease status and log CRP in the multivariate Cox model 4 [HR for (log CRP) $*$ (peripheral arterial disease status) $= 1.75$ ($P = 0.03$); HR for log CRP $= 1.07$ ($P = 0.72$); HR for peripheral arterial disease status $= 0.91$ ($P = 0.47$)]. The likelihood ratio (LR) test for comparison of the log-likelihood statistics for the interaction model [(peripheral arterial disease status) $*$ (log CRP), peripheral arterial disease status, log CRP, and all other variables of model 4 set as covariates] and the no-interaction model (log CRP and peripheral arterial disease status and all variables of model 4 set as covariate) was performed and found to be significant ($P < 0.05$).

Power calculations for survival analysis were performed separately for each patient subgroup with the PS Power and Sample Size Calculations software package, version 2.1.30 [25]. Since available software does not support power calculation for studies in which the exposition variable is continuous, patients with a CRP value less than median CRP were classified as control group, whereas patients with a CRP value greater than or equal to median CRP were classified as the exposition group. For patients without peripheral arterial disease or with peripheral arterial disease stages I to III, the study was powered with 0.8 to detect a HR of 1.42 and of 0.9 to

detect a HR of 1.51, both with a 0.05 type I error probability. For patients with peripheral arterial disease stage IV, the study was powered with 0.8 to detect a HR of 1.5 and with 0.9 to detect a HR of 1.6, both with a 0.05 type I error probability.

For analysis of the effect of dialysis membrane type on inflammation, high-flux dialysis was defined as an ultrafiltration coefficient >20 mL/hour/mm Hg and low-flux dialysis was defined as an ultrafiltration coefficient <20 mL/hour/mm Hg. Good biocompatibility was assumed for polysulfone, polyamide, and Biomembrane™ filters, whereas low biocompatibility was assumed for Hemophan® and cellulose acetate filters [26, 27]. A total of 52.2% of the patients were dialysed with polysulfone, 21.4% with Hemophan®, 11.6% with polyamide, 10.9% with Biomembrane™, and 4.0% with cellulose acetate. The distribution of membrane materials among patients with and without peripheral arterial disease stage IV did not differ significantly (Table 1) (χ^2 test, $P > 0.05$).

Statistical analysis was performed with the SPSS® version 11.5 software package (Chicago, IL, USA).

RESULTS

CRP quartiles and dialysis membrane type

CRP values were not normally distributed. Log_{10} transformation was performed to achieve normal distribution.

In our study population ($N = 445$), mean CRP was 13.25 ± 15.7 mg/L (median = 7.5, range = 0.16–96.7). CRP in quartile 1 was 0.16 to 3.69 mg/L, 3.7 to 7.5 mg/L in quartile 2, 7.51 to 17.35 mg/L in quartile 3, and 17.36 to 96.7 mg/L in quartile 4. In patients with peripheral arterial disease stage IV ($N = 190$), CRP in quartile 1 was 0.16 to 4.27 mg/L, 4.67 to 8.6 mg/L in quartile 2, 8.65 to 19.76 mg/L in quartile 3, and 20.03 to 93.08 mg/L in quartile 4. In patients without peripheral arterial disease or with peripheral arterial disease stages I to III ($N = 255$), CRP in quartile 1 was 0.17 to 3.09 mg/L, 3.24 to 6.53 mg/L in quartile 2, 6.58 to 14.6 mg/L in quartile 3, and 14.66 to 96.7 mg/L in quartile 4.

The use of low- and high-flux dialysers and of biocompatible membranes was evenly distributed across the CRP quartiles in the complete collective and in both subgroups. In addition, mean CRP values did not differ significantly between filter categories, though there was a trend for lower CRP values in patients dialysed with a high-flux (patients without peripheral arterial disease stage IV, CRP 9.6 ± 10.3 mg/L; patients with peripheral arterial disease stage IV, CRP 11.4 ± 11.2mg/L) or a biocompatible membrane (patients without peripheral arterial disease stage IV, CRP 10.9 ± 13.1 mg/L; patients with peripheral arterial disease stage IV, CRP 12.3 ± 12.5 mg/L) in comparison with patients dialysed with a low-flux (patients without peripheral arterial disease stage IV, CRP 12.9 ± 15.9 mg/L; patients with peripheral arterial disease stage IV, CRP 14.9 ± 15.9 mg/L) or a poorly biocompatible membrane (patients without peripheral arterial disease stage IV, CRP 13.2 ± 15.5 mg/L; patients with peripheral arterial disease stage IV, CRP 16.4 ± 17.7 mg/L).

Anthropometric variables by CRP quartiles

Significant differences between the CRP quartiles were observed in the male/female ratio and in smoking history only. In the upper quartiles, there were more males (65.8% vs. 37.8% males in quartile 4 and 1, respectively) ($P < 0.001$) and patients with a smoking history ($P = 0.04$, degrees of freedom = 6) than predicted by χ^2 analysis.

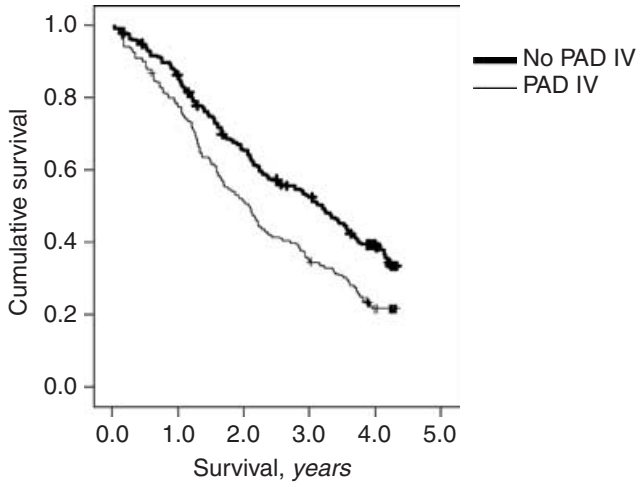
Anthropometric variables by gender

In a separate analysis of males and females, there was a trend for more males in the upper CRP quartiles having a smoking history, which was statistically not significant [for females ($\chi^2 = 3.7$, df = 3, $P = 0.30$) and for males ($\chi^2 = 0.42$, df = 3, $P = 0.94$)]. However, more females in the higher CRP quartiles had a history of cerebral ischemia ($\chi^2 = 8.35$, df = 3, $P = 0.039$). In addition, females in the higher CRP quartiles had a statistically significant shorter history of diabetes (ANOVA F = 3.02, df = 3, $P = 0.031$) and were older at diagnosis of diabetes (ANOVA F = 3.5, df = 3, $P = 0.017$). All other variables showed no difference between the CRP quartiles when analyzed by gender.

Anthropometric variables and survival by peripheral arterial disease status

As expected, patients with peripheral arterial disease stage IV had more risk factors for atherosclerosis and more frequently had coronary artery disease than patients without peripheral arterial disease or with less severe peripheral arterial disease. In the comparison of patients with peripheral arterial disease stage IV with patients without peripheral arterial disease or with peripheral arterial disease stages I to III, significant differences were observed in several variables (Table 1). Importantly, male gender, coronary artery disease, smoking history, and use of platelet aggregation inhibitors were more frequent in the group of patients with peripheral arterial disease stage IV. Beta blockers were used more frequently in patients without peripheral arterial disease or with peripheral arterial disease stages I to III. Also, duration of prior dialysis therapy and of diabetes at study inclusion was higher in the peripheral arterial disease stage IV group.

In a separate analysis of patients with peripheral arterial disease stage IV ($N = 190$) and of patients with no peripheral arterial disease or with peripheral arterial disease stages I to III ($N = 255$), significant differences between CRP quartiles were observed in gender and



Numbers at risk

Time	0	1	2	3	4
No PAD IV	255	215	161	126	64
PAD IV	190	146	97	65	25

Fig. 1. Kaplan-Meier analysis of survival on hemodialysis therapy by peripheral arterial disease (PAD) status ($N = 445$). Log rank = 13.4, $P = 0.0003$.

smoking history only. In both subgroups, males were more frequent ($P < 0.01$) and patients more frequently had a smoking history ($P < 0.05$) in the upper CRP quartiles. There were no other differences between CRP quartiles when analyzed by peripheral arterial disease stage.

Importantly, there was a significant difference in survival when comparing the peripheral arterial disease groups. From study inclusion onwards, patients with peripheral arterial disease stage IV had a mean survival of 2.24 ± 1.39 ($N = 147/190$ deaths), and patients without peripheral arterial disease stage IV had a mean survival of 2.67 ± 1.38 years ($N = 158/255$ deaths) ($P = 0.001$) (Table 1). The increase in risk for mortality in peripheral arterial disease stage IV patients was confirmed in Kaplan-Meier (Fig. 1) and Cox regression analysis [multivariate HR (Cox model 4, excluding log CRP) for peripheral arterial disease status (peripheral arterial disease stages I to III and no peripheral arterial disease set as reference variable) = 1.55, 95% CI 1.21 to 1.98, $P < 0.001$].

Morbidity and mortality by CRP quartiles

Presence or history of coronary artery disease, myocardial infarction, coronary intervention, peripheral arterial disease, and cerebral ischemia was evenly distributed amongst the CRP quartiles ($P > 0.05$) (Table 2).

Of the 445 patients, 305 (68.5%) had died at the final examination. Mean follow-up time for patients with an event, defined as all-cause mortality, was 1.84 years (±1.13 years), and 3.90 years (±0.77 years) for patients without an event. An event occurred significantly more frequently in patients in the upper CRP quartiles [quartile 1, 68 (61.3%) events; quartile 2, 71 (64.0%) events; quartile 3, 80 (72.1%) events; quartile 4, = 86 (77.5%) events] ($\chi^2 = 8.35$, df = 3, $P = 0.039$).

The causes of death, as determined by review of the patient chart and interview of the dialysis physician, are shown in Table 2. One hundred thirteen (37.1%) of all mortalities were of unknown cause or the cause could not definitely be determined. There were no significant differences between CRP quartiles in categories of death causes.

Clinical characteristics of patients with and without mortality

At study inclusion, patients had received a mean of 2.55 years (±2.1 years) of dialysis therapy. There was no significant difference by two-sided t test in prior dialysis duration between patients with (2.6 ± 2.2 years) or without an event (2.4 ± 1.9 years) ($P = 0.2$), defined as all-cause mortality. In contrast, patients with an event were older at initiation of dialysis than patients without an event (66.2 ± 8.2 years vs. 62.1 ± 9.0 years) ($P < 0.001$), more frequently had peripheral arterial disease stage IV (48.2% vs. 30.7%) ($\chi^2 = 11.99$, $P = 0.001$), a history of coronary artery disease (60.6% vs. 50.4%) ($\chi^2 = 3.95$, $P = 0.047$), myocardial infarction (33.4% vs. 16.5%) ($\chi^2 = 13.0$, $P < 0.001$), and cerebral ischemia (34.4% vs. 23.3%) ($\chi^2 = 5.3$, $P = 0.021$) than patients without an event. Therapy with HMG-CoA reductase inhibitors was associated with less frequent occurrence of events ($\chi^2 = 7.1$, df = 1, $P = 0.008$). All other variables showed no differences in patients with or without an event. Specifically, the number of events did not differ by gender ($\chi^2 = 0.4$, df = 1, $P = 0.53$).

Survival as determined by baseline CRP

Kaplan-Meier analysis showed a significant increase in risk for all-cause mortality with increasing CRP quartiles (Fig. 2A) (log rank statistic = 18.43, df = 3, $P = 0.0004$). In univariate and multivariate Cox regression survival analysis of the complete collective ($N = 445$), there was an increased risk for all-cause mortality with increasing CRP quartile with the lowest quartile set as the reference category (multivariate analysis by model 4, HR for CRP quartile 2 = 1.19, $P = 0.33$; HR for CRP quartile 3 = 1.67, $P = 0.004$; HR for CRP quartile 4 = 1.77, $P = 0.002$). An increase in log CRP was associated with an increase in all-cause mortality in the complete collective (multivariate HR by model 4 for log CRP = 1.5, $P = 0.002$).

CRP as a predictor of all-cause mortality in relation to peripheral arterial disease stage

In univariate analysis, the covariates defined in the **Methods** section had different effects on mortality in

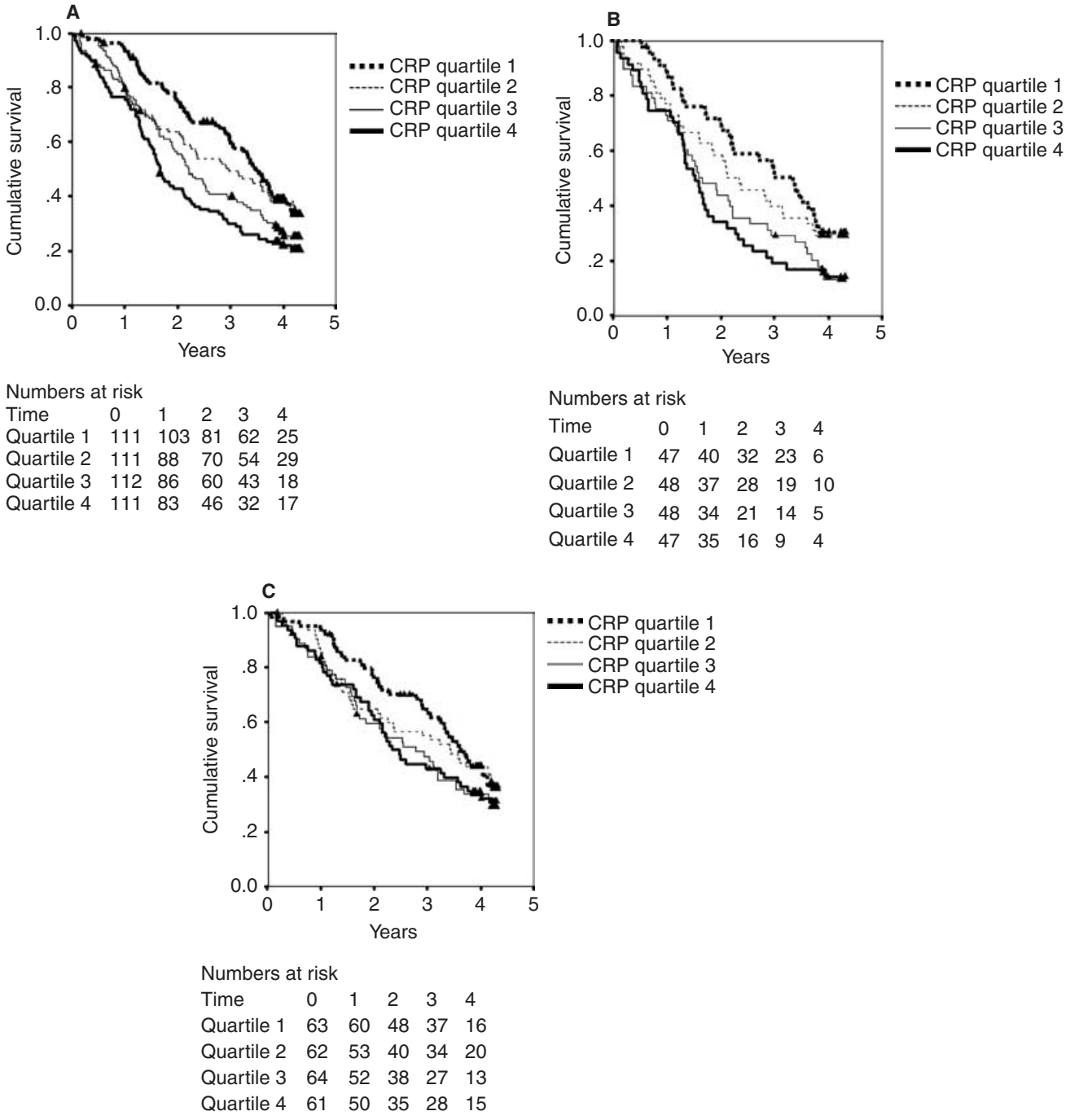


Fig. 2. Kaplan-Meier analysis of survival on hemodialysis therapy by C-reactive protein (CRP). Evaluation by quartile (*A*) ($N = 445$). End point is all-cause mortality. Patients are censored for renal transplantation ($N = 11$), lost to follow-up ($N = 2$), or if alive on December 4, 2003 ($N = 127$). Triangles represent censored patients. Log rank = 18.43, df = 3, $P = 0.0004$. (*B*) Peripheral arterial disease stage IV ($N = 190$). End point is all-cause mortality ($N = 147$). Patients are censored for renal transplantation ($N = 2$), or if alive on December 4, 2003 ($N = 41$). Triangles represent censored patients. Log rank statistic = 10.7, df = 3, $P = 0.01$. (*C*) Patients with peripheral arterial disease other than Fontaine stage IV or without peripheral arterial disease ($N = 255$). End point is all-cause mortality ($N = 158$). Patients are censored for renal transplantation ($N = 9$), lost to follow-up ($N = 2$), or if alive on December 4, 2003 ($N = 86$). Triangles represent censored patients. Log rank = 3.72, df = 3, $P = 0.29$.

patients with and without peripheral arterial disease stage IV, including log CRP (Table 3). The increase in mortality risk with increased CRP was observed only in patients with Fontaine stage IV peripheral arterial disease. CRP did not predict mortality for patients with no known peripheral arterial disease or with Fontaine stage I, II and III peripheral arterial disease (Fig. 2B and C).

Table 3. Univariate Cox proportional hazard regression analysis of survival on hemodialysis therapy, giving the hazard ratio (HR) for each of the covariates used in multivariate analysis

	No PAD, PAD stages I to III ($N = 255$)		PAD stage IV ($N = 190$)	
Covariate	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Log C-reactive protein	1.3 (0.95–1.77)	0.1	1.89 (1.34–2.66)	<0.001
Age at dialysis initiation (per year increase)	1.04 (1.02–1.06)	<0.001	1.02 (1.01–1.04)	0.04
Dialysis duration prior to study (per year increase)	0.97 (0.89–1.06)	0.52	1.08 (1.02–1.15)	0.01
Diabetes duration at study inclusion (per year increase)	1.0 (0.98–1.01)	0.57	1.0 (0.98–1.02)	0.73
Myocardial infarction (no infarction)	1.62 (1.16–2.27)	0.005	1.48 (1.05–2.08)	0.03
Coronary intervention or surgery (no intervention)	0.63 (0.4–1.01)	0.06	0.75 (0.5–1.14)	0.18
Cerebral ischemia (no ischemia)	1.35 (0.97–1.88)	0.07	1.03 (0.73–1.45)	0.86
Gender (female)	1.23 (0.9–1.67)	0.2	0.94 (0.67–1.3)	0.69
Smoking history (never-smoker)	1.04 (0.75–1.44)	0.8	1.06 (0.77–1.47)	0.73
Therapy with platelet inhibitor (no therapy)	1.04 (0.76–1.42)	0.8	0.68 (0.49–0.95)	0.02
Therapy with HMG-CoA reductase inhibitors (no therapy)	0.69 (0.48–0.99)	0.05	0.65 (0.44–0.95)	0.03
Serum albumin (per g/L increase)	0.97 (0.44–1.0)	0.09	0.97 (0.94–0.99)	0.03
Therapy with beta blocker (no therapy)	0.89 (0.63–1.27)	0.52	0.75 (0.49–1.14)	0.17
Therapy with calcium channel antagonist (no therapy)	0.91 (0.66–1.24)	0.54	0.83 (0.6–1.15)	0.17
Therapy with angiotensin-converting enzyme or angiotensin II receptor inhibitor (no therapy)	1.05 (0.75–1.46)	0.78	0.99 (0.71–1.38)	0.96
Pulse pressure (per mm Hg increase)	1.0 (0.99–1.01)	0.87	1.0 (0.99–1.01)	0.46
History of coronary artery disease (no history)	1.29 (0.94–1.77)	0.12	1.08 (0.76–1.52)	0.67
Body mass index (per unit increase)	0.96 (0.93–0.99)	0.03	0.97 (0.93–1.01)	0.55

PAD is peripheral arterial disease and HMG-CoA is 3-hydroxy-3-methylglutaryl coenzymeA.

Table 4. Multivariate Cox proportional hazard regression model of survival on hemodialysis therapy

	No PAD, PAD stages I to III ($N = 255$)			PAD stage IV ($N = 190$)		
Cox regression model	HR	95% CI	*P* value	HR	95% CI	*P* value
Log C-reactive protein model 1	1.3	0.96–1.75	0.09	2.02	1.42–2.87	<0.001
Log C-reactive protein model 2	1.25	0.91–1.72	0.18	2.01	1.4–2.88	<0.001
Log C-reactive protein model 3	1.25	0.9–1.73	0.19	1.99	1.37–2.88	<0.001
Log C-reactive protein model 4	1.36	0.97–1.93	0.08	1.75	1.18–2.6	0.006

PAD is peripheral arterial disease. The time variable is survival from study inclusion onward. All-cause mortality is defined as event. Model 1, age-adjusted; model 2, adjusted for age and hemodialysis duration at inclusion, gender, smoking history, and body mass index; model 3, adjusted for medication and covariates of model 2; and model 4, adjusted for all covariates.

Multivariate hazard ratios of log CRP for all-cause mortality for the group with peripheral arterial disease stage IV and for the group without peripheral arterial disease or with peripheral arterial disease stages I to III were 1.75 ($P = 0.006$) and 1.36 ($P = 0.08$), respectively (Table 4).

CRP as a predictor of secondary end points

Higher log CRP significantly increased risk of cardio- and cerebrovascular death in the combined collective ($N = 445$) by univariate Kaplan-Meier (for cardiac death, log rank = 17.4, df = 3, $P = 0.0006$) and multivariate analysis (for cardiac death, HR = 1.76, $P = 0.02$). The causes of 37.1% ($N = 113$) of all deaths could not definitely be determined. Classifying the undetermined events as cardiac death yields a HR for log CRP for cardiac death of 1.67 (95% CI 1.23 to 2.27, $P = 0.001$). Again, log CRP was a better predictor of cardiovascular end points in patients with peripheral arterial disease stage IV (Table 5).

Subgroup analysis of patients matched for dialysis duration and coronary artery disease

The two patient groups (peripheral arterial disease stage IV and peripheral arterial disease stages I to III or no peripheral arterial disease differed significantly in several variables (Table 1). In a substudy, we matched the two groups for prior duration of dialysis therapy and presence of coronary artery disease. In this subgroup ($N = 350$) (patient characteristics not shown), log CRP significantly predicted all-cause death in peripheral arterial disease stage IV patients (multivariate HR = 1.73, $P = 0.008$), but not in patients without peripheral arterial disease or with peripheral arterial disease stages I to III (multivariate HR = 1.27, $P = 0.24$).

Subgroup analysis of patients matched for CRP

In a further substudy, we matched the two groups for CRP values. In this subgroup ($N = 350$) (patient characteristics not shown), CRP did not differ between patients

Table 5. Hazard ratio (HR) of log C-reactive protein (CRP) for secondary end points, determined by multivariate Cox regression analysis (model 4) in the subgroups with and without peripheral arterial disease (PAD) stage IV

Combined secondary end point	No PAD, PAD stages I to III HR of log CRP ($N = 255$)	P value	PAD stage IV HR of Log CRP ($N = 190$)	P value
Cardiac death	1.57 ($N = 47$)	0.14	2.73 (1.23-6.1) ($N = 47$)	0.01
Cardiovascular death	1.47 ($N = 61$)	0.15	2.12 (1.1-4.25) ($N = 57$)	0.03
Infectious death	1.59 ($N = 24$)	0.38	1.24 ($N = 31$)	0.62
Cardiac death and death of unknown cause	1.41 ($N = 109$)	0.11	2.24 (1.36-3.7) ($N = 98$)	0.002

End points are defined as death due to cardiac causes, cardiovascular causes, or infectious complications as defined in the **Methods** section. Numbers given represent number of deaths due to the specified end point. The time variable is survival from study inclusion onwards.

with or without peripheral arterial disease stage IV (patients without peripheral arterial disease stage IV, mean CRP 12.6 ± 14.6; patients with peripheral arterial disease stage IV, mean CRP 13.9 ± 16.9, $P > 0.05$). Again, log CRP was a significant predictor of all-cause mortality only in patients with peripheral arterial disease stage IV (peripheral arterial disease stage IV, multivariate HR = 2.03, $P = 0.001$; no peripheral arterial disease, peripheral arterial disease stages I to III, multivariate HR = 1.37, $P = 0.17$).

DISCUSSION

To our knowledge, this is the first study on type 2 diabetic hemodialysis patients demonstrating that CRP has a significant predictive value for all-cause death only in patients with peripheral arterial disease in Fontaine stage IV. In patients with less severe peripheral arterial disease and in patients without peripheral arterial disease, an elevated CRP shows a trend for predicting all-cause death that is not significant or too small to be detected by our study's power. The same was shown for the secondary end points of cardiac and cardiovascular death.

Previous studies on healthy probands and hemodialysis patients have uniformly shown a significant association of death with an increased CRP at baseline, mainly due to cardiovascular causes [8, 9, 12–20]. In the studies on hemodialysis patients [12–20], CRP values and the quartile ranges were comparable to those observed in our study, suggesting a similar inflammatory state.

In contrast to our study, previous publications have not characterised the degree of peripheral arterial disease in the patient collectives studied. Currently, it is hypothesised that the increased CRP observed in dialysis patients reflects the severity of an "inflammatory state," caused in parts by dialysis itself [28], vessel inflammation due to atherosclerosis [12, 14], and oxidative stress [29]. By this theory, a high level of inflammatory activity, as reflected by elevated CRP, is associated with an increased mortality risk. However, our data may further specify this hypothesis. Elevated CRP appears to be of relevance to outcome especially in patients with a "critical mass" of severe arterial atherosclerosis. It is well known that patients with significant peripheral arterial disease have an increased risk for several adverse outcome parameters such as death with acute myocardial infarction and hospital admission [4]. Given a large surface of thus affected vasculature as encountered in peripheral arterial disease stage IV, a procoagulant state will ensue [30–34]. Activated platelets appear to play an important role in promoting leukocyte binding to affected endothelium and in progression of atherosclerosis via delivery of the chemokine regulated on activation, normal T-cell expressed and secreted (RANTES) and of platelet factor 4 [35, 36]. The additional presence of systemic inflammation, irrespective of its origin, may then be the factor "tipping the scale" toward catastrophic cardiovascular events, possibly by directly activating endothelial cells [37] or by other mechanisms. Patients with less severe atherosclerosis are less likely to have a significant surface of atherosclerotic vessels causing a relevant procoagulant state.

A recent study showed only a modest role of CRP in predicting cardiac events [9], in contrast to the results of other large studies on healthy probands [8]. In both studies, peripheral arterial disease status was not shown. The importance of peripheral arterial disease severity for mortality risk due to CRP elevation shown in our study, though in a markedly different collective, may in part explain the discrepancy of the predictive value of CRP. If the number of patients with significant peripheral arterial disease differs significantly between studies the study cohorts would be differently stratified and would thus yield varying hazard ratios for CRP.

In support of our data, deFilippi et al [13] showed that dialysis patients with elevated CRP values are more prone to suffer fatal cardiac events only if they have significant coronary artery disease, defined by elevated cardiac troponin T. An "inflammatory milieu" was thought to trigger the event in these patients, while the role of peripheral arterial disease status was not examined.

The relative role of CRP caused by the dialysis process itself or by the membranes used, by subclinical infection or by chronic vascular inflammation in predicting mortality was not the subject of examination in our study and, to our knowledge, in other studies. A study on peritoneal dialysis patients [15] had a lower median

CRP value than in our study and other studies on CRP in hemodialysis patients (2.84 mg/L vs. 7.5 mg/L). As in our study, baseline CRP at study inclusion was a significant predictor of mortality. One may hypothesize that the difference in CRP can be ascribed to a proinflammatory effect of hemodialysis membranes, since all other anthropometric data are grossly comparable in all studies. In support of this theory, we found a nonsignificant trend for higher CRP values in patients dialysed with a low flux or with a poorly biocompatible membrane. Interestingly, a higher dialysis dose and the use of high-flux filters did not influence survival in a large prospective randomized controlled study [38]. However, since the use of poorly biocompatible membranes was excluded and CRP values and peripheral arterial disease status were not reported, the effect of the dialysis process on inflammation cannot be deduced from that study. A further prospective study with well-matched patients may clarify this issue. However, the presence of subclinical infection would also be an important variable in such studies examining the role of inflammation in atherosclerosis.

In our study population, the prevalence of peripheral arterial disease was higher than in other studies examining the effect of CRP on mortality (67.4% vs. approximately 25%). We ascribe this to the fact that all the patients in our study have diabetes mellitus type 2, whereas other studies included 16% to 50% patients with diabetes [12–20]. In patients with diabetes mellitus, accelerated atherosclerosis appears to be the consequence of formation of advanced glycation end products due to hyperglycemia and oxidative stress [6], a process that may be fuelled by concomitant inflammation.

Peripheral arterial disease status was determined clinically according to Fontaine's classification. Ankle brachial index could not be determined due to vessel calcification in the majority of patients (data not shown). We cannot exclude that Fontaine classification would not correlate with angiography results in our population. However, we feel that Fontaine's classification of peripheral arterial disease stage is a practical, low-cost clinical tool for judging the severity of peripheral atherosclerotic disease.

In contrast to the data of Stenvinkel et al [17], males and females in our cohort had no statistically significant different survival when stratified for normal (CRP <3.7 mg/L) and elevated CRP (data not shown). Ethnically, the two cohorts may not be comparable and mean age at study inclusion in our cohort was higher (67.5 ± 8.2 years vs. approximately 60 years). More important, the two cohorts may have differed significantly in prevalence of peripheral arterial disease stage IV. In our population, 46.9% ($N = 115$) of males and 37.5% ($N = 75$) of females had peripheral arterial disease stage IV. Given our finding that peripheral arterial disease stage is an important factor in judging the relevance of an elevated CRP value, a significantly different male/female ratio of patients with peripheral arterial disease stage IV in the cited study may then explain the discrepancy in results.

To minimize selection bias [24], we analyzed whether more high-risk patients (e.g., higher age, history of coronary artery disease, smoking) were included in the higher CRP quartiles in the subgroup with peripheral arterial disease stage IV than in the subgroup with no or with less severe peripheral arterial disease. Indeed, in both the subgroups and in the complete collective, there were more males and patients with a smoking history in the upper than in the lower CRP quartiles, whereas in all other parameters there were no differences between quartiles in all groups. However, we do not see a relevant bias in this fact since this phenomenon was observed to a similar degree in patients with or without peripheral arterial disease stage IV.

Further, patients with or without peripheral arterial disease stage IV differed in several variables (Table 1). Possibly, the difference in effect of CRP on mortality may be ascribed to this fact. However, after post hoc matching for prior dialysis duration and presence of coronary artery disease, CRP was again associated with a significant increase in mortality in peripheral arterial disease stage IV patients only. This suggests that, while being statistically significant, the differences in anthropometric data between the subgroups may be negligible as to the effect of CRP.

Since CRP differed between subgroups, we performed post hoc matching for CRP value. Again, log CRP was a significant predictor of primary and secondary end points only in peripheral arterial disease stage IV, suggesting that the significant difference in CRP values in the total collective between the subgroups has no effect on the overall results. Nonetheless, we cannot exclude that stratification of the population may have influenced the results of our study.

A comparison of log CRP HRs between peripheral arterial disease groups is statistically not tenable. Though hazard due to an increase in log CRP is nominally higher in peripheral arterial disease stage IV patients than in patients without peripheral arterial disease stage IV, one should not conclude that mortality risk is higher. However, there was a significant effect of log CRP on survival in patients with peripheral arterial disease stage IV but not in patients without peripheral arterial disease stage IV, even though patient number was higher in the no peripheral arterial disease stage IV group than in patients with peripheral arterial disease stage IV.

Formal testing showed significant interaction between log CRP and peripheral arterial disease status, supporting our conclusion. However, in the interaction Cox regression model, peripheral arterial disease status and log CRP was found to have no significant effect on survival. Since this is in sharp contrast to our data (Figs. 1 and 2)

and that of others [12–20], it is reasonable to question whether the statistical interaction model correlates with biologic reality. The statistical analysis of biologic interaction is methodologically complex and has been regarded as error-prone [39]. In our opinion however, the highly significant, 55% difference in mortality risk between peripheral arterial disease groups in our study (multivariate analysis of effect of peripheral arterial disease status on mortality) supports our approach that these groups should not be compounded to one single group but rather placed in separate analyses, irrespective of the exposition variable of interest.

CONCLUSION

Our data suggest that the degree of atherosclerosis plays at least a similar role as that of the severity of measured systemic inflammation in predicting mortality in dialysis patients. In type 2 diabetic dialysis patients, it is the presence of severe generalized atherosclerosis that appears to prepare the ground for a high mortality risk due to elevated inflammatory parameters. Thus, before judging the predictive value of CRP for mortality or cardiovascular end points, peripheral vessel status should be determined. Given the dismal prognosis of diabetic patients with peripheral arterial disease on hemodialysis, the challenge to medical research lies in the prevention and treatment of generalized atherosclerosis complicated by inflammation.

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