

Potential cardiovascular risk factors in chronic kidney disease: AGEs, total homocysteine and metabolites, and the C-reactive protein

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Potential cardiovascular risk factors in chronic kidney disease: AGEs, total homocysteine and metabolites, and the C-reactive protein.

Background. Total homocysteine (tHcy) and advanced glycation end-products (AGEs) are implicated in the pathogenesis of vascular damage. This study aimed to investigate whether elevated serum levels of the AGEs pentosidine, N^ε-carboxymethyllysine (CML) and imidazolone; tHcy, cystathionine, methylmalonic acid (MMA), and 2-methylcitric acid (2-MCA), as well as C-reactive protein (CRP), are related to a higher risk for cardiovascular events.

Methods. A total of 232 patients with chronic kidney diseases (mean age 57.6 ± 13.1 years, 82 female and 150 male); 99 with chronic renal failure (CRF), 84 maintenance hemodialysis patients and 49 renal transplant recipients were followed for 2 years. The relationship between the parameters of interest, conventional risk factors and elevated levels of CRP with cardiovascular events was tested in all subjects by the Cox proportional hazards model.

Results. Mean serum levels of AGEs, tHcy, and of the metabolites were found to be significantly increased in all three groups compared to the healthy subjects ($P < 0.01$, respectively). Fifty-three cardiovascular events occurred during follow-up; a total of 40 patients died. Final multivariate analysis showed diabetes (RR 2.06, 95% CI 1.17–3.60, $P = 0.013$), end-stage renal disease (ESRD) (RR 4.88, 95% CI 2.40–9.89, $P < 0.001$) and elevated CRP levels (RR 2.00, 95% CI 1.11–3.60, $P = 0.021$) as independent risk factors for cardiovascular events.

Conclusion. Data from a group consisting of patients with CRF, patients undergoing maintenance hemodialysis treatment, and renal transplant recipients provide evidence that conventional risk factors such as the presence of diabetes, ESRD, as well as elevated levels of the considered risk factor CRP, seem to play a more important role for cardiovascular outcome

in patients with chronic kidney disease than elevated levels of AGEs, tHcy, and related metabolites. The evidence suggests that routine CRP measurement can be recommended in cases of chronic renal insufficiency.

Atherosclerotic vascular complications are the major cause of morbidity and mortality in patients with chronic kidney disease of different stages. The multifactorial origin of the cause is not yet well understood [1, 2]. The association between conventional risk factors, a chronic uremic state together with malnutrition and chronic inflammation seems to be a major source of oxidant stress [3, 4] playing a role in the pathogenesis of vascular damage. Advanced glycation end-products (AGEs) are involved by acting with receptors leading to the production of free oxygen radicals followed by the release of cytokines [5]. Based on the chemical pathway of AGE formation, it is supposed that patients suffering from diabetes mellitus are particularly prone to increased risk due to AGE accumulation [5, 6]. AGEs have been found to be colocalized in arterial and cardiac tissue as well as in atherosclerotic lesions of uremic patients [6, 7]. While the formation of the AGEs pentosidine and N^ε-carboxymethyllysine (CML) is closely related to oxidative stress-induced damage [8], imidazolone is a condensation product of proteins and the nonoxidatively formed 3-deoxyglucosone [6], thus can be suitably used as a marker of the nonoxidative pathway of AGE formation. The relationship between AGE-modified peptides [9] and AGEs, which accumulate with deteriorating renal function [10, 11], and cardiovascular complications, is still in discussion. The influence of nutritional status and conditions of chronic inflammation, as expressed by elevated levels of C-reactive protein (CRP) found in chronic renal failure (CRF) [4, 11–13], remains open. The same is true for the significance of increased levels of total homocysteine (tHcy), which are suggested as being a new

Key words: chronic renal failure, end-stage renal disease, hemodialysis, renal transplantation, cardiovascular risk, total homocysteine, advanced glycation end-products, C-reactive protein.

Received for publication May 13, 2003
and in revised form October 31, 2003, and January 19, 2004
Accepted for publication February 16, 2004

independent risk factor for atherosclerotic vascular disease [14], especially in patients with chronic renal failure or end-stage renal disease (ESRD) [15–17]. Homocysteine acts as a prooxidant agent via the autooxidation of free thiols leading to superoxide production [18] as well as through molecular targeting [19]. Its increase in uremia is additionally enhanced by vitamin malnutrition and functional deficiencies of the vitamin B₁₂, folic acid, and vitamin B₆ [15].

The aim of this prospective study was to investigate whether increased levels of AGEs, tHcy and its metabolites, and the CRP, are linked to a higher risk for atherothrombotic events in patients with chronic renal disease.

METHODS

Study population

A total of 232 patients with chronic kidney diseases comprising 99 patients with CRF before starting dialysis treatment (67 nondiabetics and 32 diabetics), 84 patients on maintenance hemodialysis treatment (53 nondiabetics and 31 diabetics) and 49 patients after renal transplantation (42 nondiabetics and seven diabetics) were included in the study. The 70 diabetics consisted of 67 type 2, three type 1 (two in hemodialysis and one in CRF). Reference ranges of AGEs, tHcy, and metabolites were calculated using the sera of 51 healthy volunteers (30 women and 21 men, mean age 41.7 ± 14.4 years) as controls. All of them had normal renal function as reflected by creatinine Cockcroft clearance within the normal range. None was acutely ill or revealed clinical signs of a chronic disease state, including atherothrombotic vascular changes of any type. The controls received no vitamin supplementation for at least three months. Material presented previously [20] was used in this study, changes in the numbers of patients are due to lack of certain data. All patients who checked into our dialysis unit or the outpatient department (including renal transplant recipients) within the 4-week period of recruitment, and who were expected to have the follow-up treatment in our department, were asked to participate in the study. Each participant provided written informed consent. By the end of the recruitment period, the study group was representative of the clinical population in our department; representing 83% of our intermittent hemodialyzed patients ($N = 101$), nearly 50% of our CRF patients as well as 15% of the renal transplant recipient patients routinely seen in the outpatient department.

In this study, patients were classified as having CRF when the serum creatinine level was higher than $150 \mu\text{mol/L}$; the mean duration of CRF was 46.0 ± 54.6 months (median 35 months, ranging from 1 to 274 months). Dialysis treatment was performed three times a week for 3.5 to 5 hours. Forty-nine patients were

dialyzed using low-flux cellulosic membranes and 35 patients with high-flux synthetic membranes (polycarbonate or polyamide). The mean time on dialysis was 37 ± 30 months (median 29 months, ranging from 4 to 188 months). After each dialysis session, the patients were treated with oral vitamin supplementation, consisting of vitamin B₆ 10 mg, cobalamin 6 μg , folic acid 1 mg, and biotin 0.3 mg. In the renal transplant recipients, the mean time after transplantation at baseline was 36.4 ± 17 months (median 17 months, ranging from 1 to 172 months). None of the CRF or renal transplant recipient patients received vitamin B₆, vitamin B₁₂, or folic acid supplementation. Antihypertensive treatment consisted mainly of angiotensin-converting enzyme (ACE) inhibitors (92% in CRF, 81% in hemodialysis patients, and 37% in renal transplant recipients), β receptor blockers (45%, 65%, and 40%, respectively), and calcium antagonists (18%, 27%, and 85%, respectively). Lipid-lowering drugs were used in 28% of CRF, 21% of hemodialysis patients, and 37% of renal transplant recipients. A dose of 100 mg/day aspirin was given in 17% of CRF, 35% of hemodialysis patients, and 16% of renal transplant recipients.

Renal diseases

Renal diseases in the different groups were classified for glomerulonephritis (26.3% in CRF/27.4% in hemodialysis patients/55.1% in renal transplant recipients), diabetic nephropathy (16.2%/31%/6.1%), interstitial nephritis (22.2%/23.8%/4.1%), polycystic kidney disease (3%/10.7%/8.2%), and hypertensive kidney disease (2%/4.8%/2%) as well as other causes (30.3%/7.1%/24.5%).

History of cardiovascular events/diabetes mellitus/smoking behavior

The presence of cardiovascular disease at baseline was defined if at least one of the following events occurred before the time of entry (records of former hospitalization and of outpatient clinics were used): acute myocardial infarction due to clinical and ECG or laboratory changes, angina pectoris based on clinical characteristics, cerebrovascular diseases, including hemorrhagic or occlusive stroke or transitory ischemic events, verified by computed tomography (CT) and/or the course of neurologic disorders. Peripheral arterial occlusive disease (PAOD) was recorded if patients had at a minimum strength-dependent pain leading to a maximum walking distance of below 200 m (corresponding to Fontaine's stage IIb of PAOD), ischemic rest pain (Fontaine's stage III), or ulcers of the lower extremity due to macrovascular disease (stage IV). Diabetes was classified according to the recommendations of the committee on the diagnosis and classification of diabetes mellitus in 1997 [21]. The definition of diabetes in this study, however, included a

requirement for a minimum of 2 years' duration of the disease.

Parameters of nutritional status

The body mass index (BMI) (kg/m^2) as well as the serum levels of albumin, cobalamin, and folic acid were used as markers of nutritional status.

Follow-up

Follow-up ended with the occurrence of cardiovascular events, initiation of dialysis treatment (excepting hemodialysis), renal transplantation (only hemodialysis group), a change of treatment location, or death. The presence of cardiovascular events was defined if any event of acute myocardial infarction, angina pectoris, or cerebrovascular disease, according to the criteria mentioned above, occurred. All participants had at least one ECG measurement per year. The end point of PAOD was diagnosed as described above or if a preexisting stage of PAOD had to be upgraded. Two cases of death following fatal cardiac failure in the hemodialysis group were classified as acute myocardial infarction because the autopsy revealed the total occlusion of at least one of the main coronary arteries.

Laboratory analysis

Blood samples were drawn after an overnight fasting; the serum samples were immediately centrifuged (3000 rpm, 4°C) and cooled within 1 hour and stored at -80°C until analysis was performed. Total pentosidine (free and protein-bound) was measured using the high-performance liquid chromatography (HPLC) assay described by Miyata et al [10]. The modifications used in our lab have been described elsewhere [20]. The intra- and interassay coefficients of variation (CV) were $<3\%$ and $<6\%$, respectively. CML serum concentrations were determined with an intra- and interassay CV of $<5\%$ and $<7\%$ by a competitive enzyme-linked immunosorbent assay (ELISA) using streptavidine-coated microtiter plates (Roche Diagnostics GmbH, Penzberg, Germany). Imidazolone was measured as described for the CML-ELISA, except for changes as previously described [20], with an intra- and interassay CV of $<4\%$ and $<8\%$, respectively. The imidazolone antibody was prepared and characterized as already published [6]. Imidazolone concentrations were expressed as arbitrary units of imidazolone (AU/L). Serum tHcy (free and protein-bound) and its metabolites cystathionine, methylmalonic acid (MMA) and 2-methylcitric acid (2-MCA) were measured by gas chromatographic-mass spectrometric (GC-MS) assay using stable isotope dilution as already described [22]. The intra-assay and interassay CV for tHcy are 3.9% and 4.5%, for cystathionine 1.8%/7%, for MMA 1.3%/9.1%, and for total 2-MCA (2-MCA_{total})

2.6%/10%, respectively. Serum cobalamin and folate were determined by luminescence assay (Bayer Co., Chiron, Leverkusen, Germany) using a purified intrinsic factor and a purified folate binding protein. CRP was determined using a turbidimetric immunoassay (Wako Chemicals, Neuss, Germany) with a detection limit of 2 mg/L. Parameters such as lipid levels, creatinine, and albumin were determined using automated standardized laboratory techniques.

Statistical methods

The results are given as means with standard deviations (mean \pm SD) and medians. The Mann-Whitney U test was used to compare differences between two independent groups, the Spearman rank correlation test for estimating relationships between variables. A P value < 0.05 was considered as statistically significant, with the exception of the relationships between variables (P value < 0.001). The associations between the potential risk factors and cardiovascular events in the total group are described by hazard ratios [denoted as relative risk (RR)] and the corresponding 95% CI. Patients were separated into two groups above and below the cutoff levels (medians in the total group) of the risk factors. Smokers, diabetes mellitus patients and patients with a past history of cardiovascular events were compared to those without these risk factors. Hemodialysis and renal transplant recipients were compared to CRF patients used as a reference category. The categorization of the continuous variables was necessary due to a nonlinear log hazard in the covariates of interest [23]. Relative risk for cardiovascular events was calculated by univariate following stepwise multivariate Cox regression analysis. Due to the limited number of patients, only variables on a significance level of $P \leq 0.20$ in the univariate analysis were entered in the initial multivariate model. Covariates with $P < 0.10$ were included in the final multivariate model. In addition, chi-squared statistics were used. Statistics were done using the software Statistical Package of Social Science (SPSS 10.0, 2000) (SPSS Inc., Chicago, IL, USA).

RESULTS

Biochemical and clinical characteristics of subgroups at baseline (selected parameters)

Table 1 shows the characteristics of all groups. BMI, diastolic blood pressure, CRP ($P < 0.001$, respectively), and high-density lipoprotein (HDL) cholesterol ($P = 0.003$) were significantly lower in hemodialysis compared with CRF and creatinine levels were significantly higher ($P < 0.001$, respectively) (Table 1). Albumin, HDL cholesterol levels, and diastolic blood pressure in hemodialysis patients were significantly lower as compared with renal transplant recipients ($P \leq 0.001$, respectively); age, CRP, and creatinine were each increased

Table 1. Characteristics of 99 patients with chronic renal failure (CRF), 84 hemodialysis patients and 49 patients after renal transplantation

	Total	CRF	Hemodialysis	Renal transplant recipients	Normal range ^a
Gender male/female	150/82	65/34	50/34	35/14	
Diabetes/smokers	70/42	32/22	31/9	7/11	
History of cardiovascular events	79	20	42	17	
Age years					
Mean \pm SD	57.6 \pm 13.1	58.3 \pm 12.9 ^b	61.1 \pm 12.5 ^b	50.2 \pm 11.8 ^c	
Median	58.6	59.2	61.5	54.0	
(min-max)	(24-87)	(33-83)	(24-87)	(25-71)	
Body mass index kg/m ²					
Mean \pm SD	26 \pm 4	28 \pm 5 ^{b,d}	25 \pm 4 ^e	25 \pm 3 ^e	20-25
Median	26	27	25	25	
(min-max)	(15-47)	(19-47)	(15-39)	(18-35)	
Creatinine μ mol/L					
Mean \pm SD	482 \pm 318	381 \pm 213 ^{b,d}	794 \pm 220 ^{b,e}	150 \pm 66 ^c	80-95
Median	453	324	745	142	
(min-max)	(64-1481)	(119-1107)	(397-1481)	(64-371)	
C-reactive protein mg/L					
Mean \pm SD	17.2 \pm 28.2	23.5 \pm 35.9 ^{b,d}	16.2 \pm 22.1 ^{b,e}	6.3 \pm 12.1 ^c	<5.0
Median	6.7	9.2	5.0	3.2	
(min-max)	(1.0-236.9)	(4.1-236.9)	(1.0-96.0)	(1.0-84.5)	
Albumin g/L					
Mean \pm SD	39.2 \pm 5.3	38.7 \pm 5.1 ^b	38.0 \pm 5.0 ^b	42.5 \pm 5.0 ^c	35-55
Median	39.7	39.6	38.3	42.6	
(min-max)	(20.1-65.1)	(20.1-55)	(25.3-54.2)	(31.5-65.1)	
Low-density lipoprotein mmol/L					
Mean \pm SD	3.5 \pm 1.2	3.7 \pm 1.2	3.4 \pm 1.1	3.3 \pm 1.2	<3.5
Median	3.5	3.6	3.4	3.2	
(min-max)	(0.88-8.11)	(0.9-8.1)	(0.9-7.6)	(0.9-5.2) ^d	
High-density lipoprotein mmol/L					
Mean \pm SD	1.3 \pm 0.8	1.2 \pm 0.4 ^{b,d}	1.1 \pm 0.4 ^{b,e}	1.7 \pm 1.6 ^c	>1.1
Median	1.2	1.2	1.0	1.4	
(min-max)	(0.36-10.8)	(0.5-2.7)	(0.4-2.3)	(0.5-10.8)	
Triglycerides mmol/L					
Mean \pm SD	2.2 \pm 1.5	2.2 \pm 1.3	2.1 \pm 1.3	2.6 \pm 2.0	<2.29
Median	1.9	2.0	1.7	1.9	
(min-max)	(0.34-11.1)	(0.3-8.2)	(0.4-6.7)	(0.5-11.1)	
Systolic blood pressure mm Hg					
Mean \pm SD	139 \pm 20	140 \pm 19	140 \pm 22	137 \pm 17	<140
Median	140	140	143	134	
(min-max)	(88-192)	(100-192)	(88-186)	(90-176)	
Diastolic blood pressure mm Hg					
Mean \pm SD	81 \pm 11	83 \pm 11 ^d	77 \pm 11 ^{b,e}	83 \pm 11 ^d	<90
Median	80	85	77	84	
(min-max)	(38-109)	(51-105)	(38-100)	(49-109)	
Cobalamin pmol/L					
Mean \pm SD	341 \pm 170	352 \pm 201	347 \pm 146 ^b	310 \pm 140 ^d	222-814
Median	311	314	312	266	
(min-max)	(110-1476)	(110-1476)	(139-1040)	(151-811)	
Folate nmol/L					
Mean \pm SD	25.5 \pm 23.2	19.6 \pm 9.8 ^d	37.0 \pm 34.4 ^{b,e}	19.2 \pm 9.7 ^d	6.8-38.6
Median	19.2	16.9	27.4	16.8	
(min-max)	(4.7-178)	(4.7-52.0)	(8.4-178.0)	(7.5-52.0)	

^aNormal ranges referred to healthy, middle-aged men and women without cardiovascular risk profile.^b $P \leq 0.001$ compared to renal transplant recipient, $P = 0.039$ for cobalamin.^c $P < 0.001$ compared to chronic renal failure and hemodialysis, $P \leq 0.023$ for high-density lipoprotein cholesterol.^d $P \leq 0.001$ compared to hemodialysis, $P = 0.003$ for high-density lipoprotein cholesterol.^e $P \leq 0.001$ compared to chronic renal failure, $P = 0.003$ for high-density lipoprotein cholesterol.

($P < 0.001$, respectively). In addition, renal transplant recipients revealed significantly higher albumin levels ($P < 0.001$) and HDL cholesterol levels ($P = 0.023$) as compared with CRF and hemodialysis patients; age, creatinine, and CRP were significantly lower ($P < 0.001$, respectively). BMI in renal transplant recipients was lower as compared with CRF ($P = 0.001$, respectively).

AGEs, tHcy, related metabolites, folate, and vitamin B₁₂

At baseline, the mean serum levels of the AGEs were found to be significantly increased above the reference interval (healthy subjects) in all three groups ($P < 0.001$) (Fig. 1). The hemodialysis group showed the highest levels for all AGEs ($P < 0.001$ vs. CRF and renal transplant recipients). Compared to CRF, CML in renal

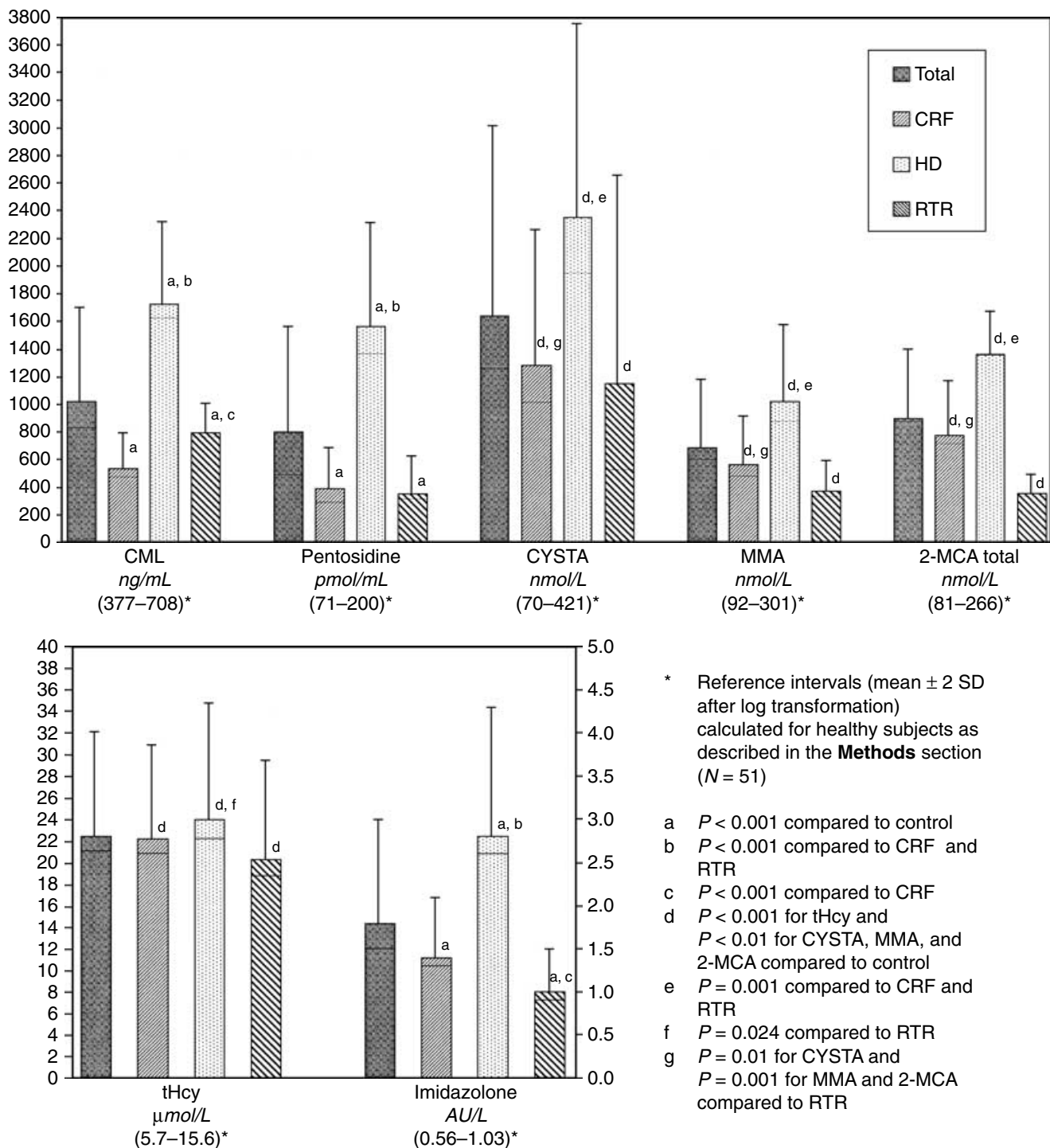


Fig. 1. Serum levels of advanced glycation end products (AGEs), total homocysteine (tHcy), and metabolites at baseline of 99 patients with chronic renal failure (CRF), 84 hemodialysis patients (HD), and 49 patients after renal transplantation (RTR), mean, SD, median (—), respectively. Abbreviations are: CML, N^ε-carboxymethyllysine; CYSTA, cystathionine; MMA, methylmalonic acid; 2-MCA_{total}, total 2-methylcitric acid.

transplant recipients was significantly increased and imidazolone significantly decreased ($P < 0.001$). Mean serum levels of tHcy and of the metabolites were significantly increased, compared to controls ($P < 0.001$ for tHcy, $P < 0.01$ for cystathionine, MMA, and 2-MCA).

Hyperhomocysteinemia $>15 \mu\text{mol/L}$ was present in 82% ($N = 81$) of the CRF patients, 89% ($N = 75$) of the hemodialysis patients and 63% ($N = 31$) of the renal transplant recipients. Hemodialysis patients had significantly higher levels of cystathionine, MMA, 2-MCA, and

Table 2. Significant relationships between selected parameters according to Spearman-Rho test (total group, $N = 232$), R values are given, $P < 0.001$, respectively

	Albumin	CML	Imidazolone	Pentosidine	tHcy	Cystathionine	MMA	2-MCA
Age	-0.27	NS	0.35	0.34	NS	0.25	0.34	0.33
Albumin	—	NS	-0.31	NS	NS	NS	NS	NS
Body mass index		-0.38	NS	-0.30	NS	NS	NS	NS
C-reactive protein	-0.30	NS	NS	NS	NS	NS	NS	NS
Cobalamin	NS	NS	NS	NS	NS	NS	NS	NS
Folic acid	NS	0.29	0.30	0.27	-0.30	NS	NS	0.27
Creatinine	NS	0.60	0.69	0.73	0.32	0.53	0.55	0.79
CML	NS	—						
Imidazolone	-0.31	0.57	—					
Pentosidine	NS	0.82	0.65	—				
tHcy	NS	NS	NS	0.27	—			
Cystathionine	NS	0.47	0.49	0.56	0.40	—		
MMA	NS	0.61	0.57	0.68	0.37	0.60	—	
2-MCA	NS	0.63	0.69	0.75	0.36	0.63	0.66	—

Abbreviations are: CML, N^ε-carboxymethyllysine; tHcy, total homocysteine; MMA, methylmalonic acid; 2-MCA_{total}, total 2 methylcitric acid.

folic acid as compared to CRF and renal transplant recipients ($P = 0.001$, respectively). tHcy and cobalamin in hemodialysis patients were significantly increased as compared with renal transplant recipients ($P = 0.024$ and $P = 0.039$, respectively), whereas CRF revealed significantly higher levels of cystathionine ($P = 0.01$), MMA, and 2-MCA ($P = 0.001$, respectively) in comparison to renal transplant recipients (Table 1) (Fig. 1).

Relationships within selected variables

Significant parameter correlations in the total group are shown in Table 2. No significant relationships between tHcy and CRP, BMI or albumin levels could be found in the total group, whereas in hemodialysis patients, tHcy was positively correlated with albumin ($R = 0.369$, $P = 0.001$). Expecting a negative correlation of imidazolone with albumin (Table 2), no significant correlation between albumin and the AGEs could be found in the total group. In hemodialysis patients, beside pentosidine ($R = 0.29$), CML was correlated with tHcy ($R = 0.37$, $P < 0.001$, respectively). No significant correlation of AGEs with HDL, low-density lipoprotein (LDL) and triglycerides could be found in the total group or in any of the subgroups.

Parameters in hemodialysis patients with low-flux vs. high-flux membrane dialysis

With the exception of fibrinogen (median 3.8 in high flux vs. 4.4 in low-flux, $P = 0.010$) and creatinine levels (823 $\mu\text{mol/L}$ vs. 724 $\mu\text{mol/L}$, $P = 0.009$), none of the parameters showed any difference between high- and low-flux membrane dialyzed patients.

Parameters in patients with vs. without history of cardiovascular events

In CRF patients, no significant differences between patients with a history of cardiovascular events compared to those without were found for all baseline data, including markers of nutrition and the levels of AGEs, tHcy, and metabolites, with the exception of pentosidine (median

435 pmol/mL in cardiovascular events vs. 265 pmol/mL in noncardiovascular events, $P = 0.032$) and MMA (655 nmol/L vs. 473 nmol/L, $P = 0.044$). In hemodialysis patients, albumin (median 37.5 g/L in cardiovascular events vs. 38.9 g/L in noncardiovascular events, $P = 0.014$), tHcy (20.1 $\mu\text{mol/L}$ vs. 22.9 $\mu\text{mol/L}$, $P = 0.013$), imidazolone (2.7 AU/L vs. 2.2 AU/L, $P = 0.015$) as well as CRP (11.0 mg/L vs. 4.9 mg/L, $P = 0.001$) were found to be significantly different. The renal transplant recipient group revealed significant differences for age (median 57.0 years in cardiovascular events vs. 48.5 years in noncardiovascular events, $P = 0.021$) and HDL cholesterol (1.53 mmol/L vs. 1.30 mmol/L, $P = 0.016$).

Follow-up/dropouts of the total group

Median follow-up time was 26 (1 to 62) months. A total of 56 patients (20 female and 36 male, mean age 59.4 ± 11.8 years) dropped out of the study because of renal transplantation ($N = 7$) or change of dialysis center ($N = 2$) in the hemodialysis group or due to dialysis initiation in the CRF ($N = 42$) and renal transplant recipient group ($N = 5$). Baseline characteristics were the following: CRP (median 7.4 mg/L in dropouts vs. 6.1 mg/L in remaining subjects, $P = 0.039$), urea (24.8 mmol/L vs. 19.8 mmol/L, $P < 0.001$), tHcy (24.5 $\mu\text{mol/L}$ vs. 20.0 $\mu\text{mol/L}$, $P = 0.001$), diastolic blood pressure (87 mm Hg vs. 80 mm Hg, $P = 0.016$), LDL cholesterol (3.8 mmol/L vs. 3.2 mmol/L, $P = 0.002$), and percentage of prior cardiovascular events (22.8% vs. 37.7%, $P = 0.034$).

Total and cardiovascular mortality

A total of 40 patients died during the follow-up period. Twenty-four patients died from acute myocardial infarction; 19 in the hemodialysis group, four in the CRF group, and one in the renal transplant receipt group. Two cases of death in hemodialysis patients following fatal cardiac failure were classified as acute myocardial infarction, because the autopsy revealed the total occlusion of at least one of the main coronary arteries. Seven patients died of cerebrovascular complications; two patients in CRF

and five in the hemodialysis group. Nine patients died of other causes, two outside of the hospital without a known cause.

Cardiovascular events during follow-up

Fifty-three cases of cardiovascular events, including cardiovascular death, occurred during the follow-up (total group). Thirty (18 acute myocardial infarction, one angina pectoris, five cerebrovascular events, and six PAOD) of the 53 cardiovascular events occurred in subjects with a history of prior cardiovascular events vs. 23 in subjects without ($P < 0.001$). Fourteen of the subjects with recurrent cardiovascular events were type 2 diabetics and two were type 1 diabetics, five were smokers. In nine cases, the cardiovascular event was of the same type (five acute myocardial infarction, one angina pectoris, and three PAOD). Twenty-one deaths due to cardiovascular causes (16 due to acute myocardial infarction and five due to cerebrovascular disease) occurred in patients having a history of cardiovascular events, 11 of them had type 2 diabetes, one case with type 1 diabetes, and three were smokers. Among the 99 CRF patients, 11 had cardiovascular events (acute myocardial infarction, four; angina pectoris, one; cerebrovascular disease, five; peripheral vascular disease, one). In the 84 hemodialysis patients, 39 suffered from cardiovascular events, including acute myocardial infarction in 21 cases, angina pectoris in six, cerebrovascular disease in four as well as peripheral vascular disease in eight patients. In the 49 renal transplant recipients, one patient in each case revealed acute myocardial infarction, angina pectoris as well as cerebrovascular disease.

Diabetics and nondiabetics

In CRF, differences between diabetic and nondiabetic patients existed (median values in diabetic vs. nondiabetic, respectively) for cystathionine (1597 nmol/L vs. 812 nmol/L, $P = 0.006$), diastolic blood pressure (74 mm Hg vs. 90 mm Hg, $P < 0.001$), HDL cholesterol (1.0 mmol/L vs. 1.2 mmol/L, $P = 0.005$), LDL cholesterol (3.2 mmol/L vs. 3.7 mmol/L, $P = 0.024$), and imidazolone concentrations (1.6 AU/L vs. 1.2 AU/L, $P = 0.007$). Hemodialysis patients with diabetes had lower levels of tHcy (21.4 μ mol/L vs. 22.9 μ mol/L, $P = 0.007$), 2-MCA (1269 nmol/L vs. 1434 nmol/L, $P = 0.002$), creatinine (733 μ mol/L vs. 804 μ mol/L, $P = 0.019$), CML (1517 ng/mL vs. 1668 ng/mL, $P = 0.036$), and pentosidine (1170 pmol/mL vs. 1503 pmol/mL, $P = 0.027$) but higher triglyceride levels (2.3 mmol/L vs. 1.5 mmol/L, $P = 0.015$) compared with nondiabetics. In renal transplant recipients, none of the parameters showed any difference between diabetic and nondiabetic patients.

Aspirin, CRP, and cardiovascular events

No significant differences in CRP levels between patients treated with aspirin medication and those with-

Table 3. Adjusted relative risk estimates for cardiovascular events ($N = 53$) during a median follow-up of 26 months in 232 patients with chronic kidney diseases resulting from final multivariate Cox proportional hazards model

Categories of risk factors ^a	Relative risk (RR) estimate (95% CI) adjusted final model ($P < 0.10$)	P value
End-stage renal disease ^b	4.88 (2.40–9.89)	<0.001
Diabetes mellitus	2.06 (1.17–3.60)	0.013
C-reactive protein >median (6.7 mg/L)	2.00 (1.11–3.60)	0.021

^aDefining relative risk compared to complementary categories.

^bHemodialysis treatment; defining relative risk compared to chronic renal failure (reference).

out could be found in any group, whereas patients with cardiovascular events during follow-up had significantly higher CRP levels compared to patients without (median 10.7 mg/L vs. 6.4 mg/L, $P = 0.003$). Thirty-five percent of patients with aspirin treatment had new cardiovascular events during follow-up compared to 19% without ($P = 0.013$).

Relationships between the variables of interest and vascular diseases during follow-up (total group)

In univariate analysis, higher values for age, CRP, CML, pentosidine, imidazolone, cystathionine, MMA, and 2-MCA at baseline (categorical variables, separated by the median values in the total group, respectively) (Table 1) (Fig. 1) and lower values of albumin, BMI, folic acid, HDL cholesterol as well as the existence of diabetes mellitus, smoking, a history of cardiovascular events, male gender, and advanced stage of renal insufficiency (hemodialysis and renal transplant recipients vs. CRF, respectively) were associated with the subsequent development of cardiovascular events ($P < 0.2$). Final multivariate Cox regression analysis showed diabetes mellitus (RR 2.06, 95% CI 1.17–3.60, $P = 0.013$), hemodialysis treatment (RR 4.88, 95% CI 2.40–9.89, $P < 0.001$) as well as elevated CRP levels (RR 2.00, 95% CI 1.11–3.60, $P = 0.021$) as correlated to increased risk for cardiovascular events (Table 3). Neither elevated levels (>median, respectively) of CML (>832 ng/mL), imidazolone (>1.50 AU/L) nor of tHcy (>21.2 μ mol/L) and its metabolites cystathionine (>1261 nmol/L), MMA (>602 nmol/L), and 2-MCA (>876 nmol/L) were significantly associated with an increased risk for cardiovascular events during follow-up. Higher pentosidine levels (>491 pmol/mL) tended to almost reach significance in the final backward model ($P = 0.06$).

DISCUSSION

In patients with chronic renal failure, risk of cardiovascular morbidity and mortality is substantially increased [1]. In addition to the well-known cardiovascular risk factors such as diabetes mellitus or ESRD, parameters such as elevated serum levels of CRP, fibrinogen, and tHcy

have been newly defined as cardiovascular risk factors [12–14, 16, 17, 24, 25]. Evidence increasingly suggests that AGEs are also potential candidates enhancing vascular complications [7, 26]. The present study tested the hypothesis that related to renal impairment, patients with chronic kidney disease, including CRF, maintenance dialysis patients as well as renal transplant recipients, are at increased risk for cardiovascular events due to the elevation of tHcy, their related metabolites cystathionine, MMA, and 2-MCA as well as of the AGEs CML, pentosidine, and imidazolone. The influence of chronic inflammation as well as malnutritional status mainly expressed by increased levels of CRP and fibrinogen and decreased serum albumin levels [3, 4, 12, 13, 24] was also tested.

As stated by Suliman et al [15], the causative role of hyperhomocysteinemia for atherothrombotic diseases in consideration of nutritional status remains unclear. In our study, 82% of CRF, 89% of hemodialysis patients, and 63% of renal transplant recipients had tHcy levels $>15 \mu\text{mol/L}$. Comparable to Suliman et al, hemodialysis patients with a history of cardiovascular events revealed lower tHcy ($P = 0.013$) and albumin levels ($P = 0.014$) compared to those without. Hemodialysis patients had the lowest mean albumin concentration ($38.0 \pm 5.0 \text{ g/L}$) of all groups. Only in hemodialysis patients, albumin correlated with tHcy ($R = 0.37$, $P = 0.001$) suggesting that malnutrition along with lower tHcy levels mainly occurs in dialyzed patients. Interestingly, in contrast to the total group, in hemodialysis patients no correlation between albumin and CRP existed, indicating that malnutrition in hemodialysis patients is not urgently related to acute phase response, respectively, caused by it, as stated by Stenvinkel et al [4] in a predialysis population study. A possible role of the metabolites cystathionine, MMA, and 2-MCA in addition to increased tHcy levels has not yet been fully clarified, although their extraordinary increase in CRF, above all in hemodialysis patients [27], as confirmed by our results (Fig. 1) is caused both by a reduced glomerular filtration rate as well as by functional deficiencies of the vitamin B₆, vitamin B₁₂, and folic acid [27, 28]. The inhibition of metabolic steps due to uremia may play a further role [28]. Metabolites and tHcy are possibly more closely linked to renal function rather than nutritional status, which might suggest a cardiovascular risk potential due to vitamin deficiency and disturbed Hcy metabolism leading to a generalized increase in thiol oxidation in renal failure [3, 18, 28]. The increased levels of the metabolites, additional to elevated tHcy in renal transplant recipients, showing a mean creatinine of only $150 \mu\text{mol/L}$, might be an indicator for this as well.

The results of the present study regarding the concentration of AGEs in chronic kidney disease confirm previous findings [10, 11]. In a preliminary study recently published [20], CRF, hemodialysis, and renal transplant recipient patients with left ventricular hypertrophy re-

vealed higher AGE levels. Only minimal data exist correlating tHcy and AGE levels in testing the hypothesis of synergistic action of both in the generation of vascular damage [22] as suggested in diabetic patients [29]. In the data presented here, pentosidine showed a constant correlation with tHcy in the total group (Table 2) as well as in hemodialysis patients. In hemodialysis patients, CML correlated additionally with tHcy, whereas imidazolone showed no correlation with tHcy in the total group or in any of the subgroups. Beyond their close association with renal function (Table 2), these results might suggest a link between oxidatively formed AGEs and tHcy levels confirming both as markers of increasing oxidative stress in CRF and hemodialysis as indicated by Himmelfarb et al [3]. Moreover, it might confirm that “carbonyl stress” results from oxidative stress [28]. The formation of glycoxidation products, such as CML and pentosidine, is dependent on the concentration of carbohydrate precursors and of reactive oxygen, the autooxidation of homocysteine to homocystine may result in such reactive oxygen species [18]. In the same manner, one would expect that the non-oxidatively formed imidazolone would show no relationship with tHcy; exactly this could be shown for all groups. However, no relationship of AGEs with CRP in the total group or in any subgroup could be determined indicating that “carbonyl stress” is not as closely related to chronic inflammation than the loss of renal function itself, leading to a decreased renal detoxification or clearance of reactive carbonyl compounds which act as AGE precursors [26]. The strong correlation of the AGEs with the creatinine level as shown for the total group (Table 2) emphasizes this. In a prospective study investigating dialyzed patients, Schwedler et al [11] showed that higher serum levels of AGEs were linked to a better survival and were associated with higher levels for creatinine, albumin and blood urea nitrogen, which was interpreted as an indicator of a better nutritional status; survival worsened when low AGEs and high CRP levels coincided. In contrast, in the hemodialysis group of our study, no significant correlation between AGEs and albumin and creatinine could be found, suggesting AGEs are not constantly linked to albumin and creatinine levels, especially under conditions of chronic hemodialysis treatment. In animal models, the increase in CML tissue levels was attributed mainly to hyperlipidemia [31], and AGEs correlated with serum triglycerides in nonuremic patients with diabetes mellitus [32]. In our study, no significant correlation between serum lipid levels and AGEs, especially CML, could be found, possibly indicating a different impact of lipids for the AGE formation in CRF and, furthermore, that AGE serum levels in CRF might not correspond to tissue levels.

Our main findings were that elevated CRP levels, diabetes, and hemodialysis treatment were associated with increased morbidity for cardiovascular events (Table 3), whereas elevated serum levels of AGEs, tHcy, or any of

the metabolites were not related to elevated cardiovascular event risk. Only for increased pentosidine could be detected a trend toward a possible association with an increased risk. These results confirm numerous previous findings in which diabetes, elevated CRP levels, and hemodialysis treatment could be demonstrated as independent risk factors for cardiovascular events, especially in chronic renal disease patients [1, 2, 13, 24, 33]. Nevertheless, it should be noted that there are some limitations to our study: for statistical reasons stated above, it was necessary to use the categorization of continuous variables. Moreover, due to the limited number of patients recruited in this single-center study, we performed median dichotomization rather than comparing quartiles leading to the possibility that only tHcy or AGE levels in the upper 20th to 25th percentile distribution could have been associated with a higher risk for cardiovascular events as shown for tHcy by others [16, 17]. Another problem might be that AGEs, tHcy, and the metabolites were measured only at baseline levels and might have been influenced by short-term changes in formation and metabolism. Furthermore, it has to be taken into account, that the hemodialysis group, which was found to have the highest tHcy concentrations within the study population, was treated with vitamin supplementation, possibly covering up any relationship between tHcy and cardiovascular events, if not preventing it. However, since ESRD did lead to a risk potentiation for cardiovascular events in our study population (Table 3) and maximum serum levels of tHcy, metabolites, and AGEs were observed in this group (Fig. 1), one can speculate that the interaction between these enhanced parameters in ESRD might play a role as well.

Himmelfarb et al [3] hypothesize oxidant stress enhanced by chronic inflammation and malnutrition as a unifying concept of cardiovascular disease in uremia. Our data are not contradictory to this concept, but in a confirmation of previous findings [11–13, 24], our results indicate a more pronounced role for inflammation itself, as expressed in increased CRP levels. Although serum levels of CRP are independent of the glomerular filtration rate, it is known that even in predialysis patients CRP levels begin to rise, potentially related to a sustained inflammatory response in CRF [4]. Since standard assays for CRP lack the sensitivity to determine levels of inflammation within the normal range of the general population, application of highly sensitive CRP (HSCRP) testing has become a tool in cardiovascular risk estimation [34]. For each quintile increase in HSCRP (0.1 to 0.69 mg/L; 0.7 to 1.1 mg/L; 1.2 to 1.9 mg/L; 2.0 to 3.8 mg/mL; and >3.8 mg/L), the adjusted risk of suffering a future cardiovascular event increased 26% for healthy American men (95% CI 11% to 44%, $P < 0.005$) and 33% for women (95% CI 13% to 56%, $P < 0.001$) [34]. In patients with CRF, especially in ESRD, elevated CRP levels, which are present in up to 65% of those patients, were also linked

to increased all-cause and cardiovascular mortality [4, 12, 13, 24]. Zimmerman et al [13] found a significant increase of all-cause and cardiovascular mortality due to increased CRP in a cohort of 280 hemodialysis patients (4.6-fold and 5.5-fold higher in the highest quartile (>15.8 mg/L) compared to the lowest (<3.3 mg/L), respectively. Agreeing with our results, Pecoits-Filho et al [35] showed a median CRP level of 6.3 mg/L in a predialysis cohort. In other studies, mean CRP levels of 18 mg/L (predialysis) and 16.3 mg/L/16.2 mg/L (hemodialysis) were shown [4, 13, 33] comparable to the mean CRP levels of our study. All results from these studies, including ours, show that CRP levels were clearly increased above the normal range of the general population [34] suggesting that a standard assay for CRP testing might be sufficient for the estimation of increased cardiovascular risk due to elevated CRP in CRF and ESRD. Evidence increasingly points to the potential of reducing CRP levels by the administration of high dosages of aspirin (at least 160 mg/day) [36, 37] and by lipid-lowering drugs such as the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors [38, 39] for both primary and secondary prevention of cardiovascular disease in patients without renal failure. Concerning therapy with aspirin, comparable to Schwedler et al [11], our study showed no benefits derived from aspirin treatment regarding CRP levels and rate of cardiovascular events. In the literature, as far as we know, no data exist as yet which have demonstrated a drug-mediated CRP reduction in patients with CRF. Our findings might be related to the use of a maximum dose of only 100 mg/day; comparable doses had no effect on CRP levels even in healthy volunteers [40]. Other possible confounders are, for example, the previous cardiovascular event rate (mainly patients with previous cardiovascular events used aspirin), and that most subjects in the hemodialysis group, showing the highest rate of cardiovascular events, were aspirin users. Prospective placebo-controlled trials would help determine the benefits of aspirin and lipid-lowering drugs in reducing the risk of future cardiovascular events in CRF and ESRD by decreasing CRP concentrations.

Our data underline that the presence of diabetes remains a cardiovascular risk factor of extraordinary importance, especially in chronic kidney disease. The presence of both chronic kidney disease and diabetes leads to the abysmal prognosis of diabetes patients when requiring dialysis treatment, as reported for type 2 diabetes [33], and is further intensified by increasing CRP levels [4, 11–13, 24].

CONCLUSION

Our data provide evidence that risk factors such as elevated CRP levels, the presence of diabetes, and the extent of ESRD seem to play a more important role for the cardiovascular outcome of patients with chronic kidney diseases than the laboratory findings of elevated serum

levels of tHcy, cystathionine, MMA, 2-MCA, or of the AGEs CML, pentosidine, and imidazolone. As evidence clearly shows that elevated CRP correlates with negative cardiovascular outcome as confirmed by this study, CRP testing can be recommended for determination of increased cardiovascular risk in patients with chronic renal insufficiency; a standard CRP assay would be sufficient.

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