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Impact of malnutrition–inflammation on the association between homocysteine and mortality

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Whether high total serum homocysteine levels (tHcy) contribute to increase mortality or offer a survival advantage in chronic hemodialysis patients remains controversial. We conducted a prospective study to determine the impact of tHcy on survival in this population with special respect to chronic inflammation–malnutrition state (CIMS). In this prospective study, 459 hemodialysis patients from 10 dialysis centers located in two regions of France were included. A number of baseline parameters were measured including tHcy and markers of CIMS. Over a mean follow-up period of 54 months, 219 deaths (47.7%) occurred, of which 114 (52%) were of cardiovascular (CV) origin. tHcy of equal to or greater than 30 μmol/l was associated with a higher risk of all-cause mortality in patients without CIMS (hazard ratio (HR): 1.55 (confidence interval (CI): 1.12–4.72)), but not in overall dialysis population or those with CIMS. When only CV mortality was considered, tHcy of equal to or greater than 30 μmol/l was associated with a higher risk in patients without (CIMS HR: 1.91 (CI: 1.23–3.23)), but not in those with CIMS. Hyperhomocysteinemia is a strong risk factor for all-cause and CV mortality in hemodialysis patients who do not present CIMS. This association might be masked in patients with CIMS.

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Dialysis patients present disproportionately high rates of atherosclerotic events and cardiovascular (CV) mortality.[1,2] A high prevalence of traditional and nontraditional CV risk factors account for the increased incidence of atherosclerotic complications in this population, although it seems difficult to assess the relevance of each factor separately. As a supplementary complication, a number of reports indicate that, contrary to the general population, some CV risk factors are strongly correlated with a decreased rate of cardiovascular events (CVE) and death.[3] Subsequently, a concept of 'reverse epidemiology' has emerged to account for the inverse association observed in dialysis patients between some so-called traditional CV risk factors of the general population and atherosclerotic disease.[3]

One of these factors is hyperhomocysteinemia (HHcy). Indeed, although some initial epidemiologic studies[4–9] have found a strong association between HHcy and increased risk of CVE and mortality in the dialysis population, a low total serum homocysteine level (tHcy) has more recently been reported to be a strong predictor of an elevated mortality risk in dialysis patients.[10–12] Nevertheless, even when these studies are concordant and include large sample population size in some cases, they are not exempt from bias and are unlikely to reflect a causal relationship. Indeed, there is no theoretical basis for an association between atherosclerosis and low tHcy levels. Therefore, a reasonable doubt concerning this reverse effect of HHcy in end-stage renal disease patients is allowed. Especially, this paradoxical association may be due to a confounding effect of inflammation and/or malnutrition, which leads to lower homocysteine levels.

We conducted a prospective multicenter study to address the role of HHcy on survival rate in hemodialysis population with respect to other known CV risk factors, and to evaluate whether the association between HHcy and mortality would eventually be altered by the presence of a chronic inflammation–malnutrition syndrome (CIMS). In this study, we included all patients on chronic hemodialysis in two regions of France in a consecutive and unselected manner.

RESULTS

Demographic characteristics of the population

The demographic, clinical, and biological characteristics of the study population are summarized in Table 1. Evidence of CIMS was found in 49.8% ($n = 228$) of the patients. The

Table 1 | Characteristics of the study population

Variable	Mean	Standard deviation	Median
Age (years)	65	13	68
Male gender	62%		
Current smoking	25%		
Past history of cardiovascular disease	45%		
Diabetes mellitus	20%		
Duration of dialysis (years)	42	35	30
Weekly dialysis session duration (h)	12.2	1.2	12
PTH (pg/ml)	230	211	158
Hemoglobin (g/100 ml)	10.8	1.4	10.9
tHcy (μmol/l)	27.7	8	26
BMI (kg/m^2)	24.2	4.2	23.9
Predialysis SBP (mmHg)	143	23	143
Predialysis DBP (mmHg)	76	12	76
Predialysis PP (mmHg)	67	17	67
K_t/V	1.47	0.33	1.43
nPCR	1.03	0.21	1.03
CRP (mg/l)	6.3	4.3	5
Serum albumin (g/l)	37	4.7	37

BMI, body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; nPCR, protein catabolic rate; PP, pulse pressure; PTH, parathyroid hormone; SBP, systolic blood pressure; tHcy, total serum homocysteine level.

Table 2 | Demographic, clinical and biological variables in patients with and without CIMS

	Patients without CIMS	Patients with CIMS	*P*-value
Age (years)	64 ± 14	66 ± 13	0.23
Male sex (%)	63	61	0.24
Duration of dialysis (years)	40 ± 37	44 ± 36	0.29
Weekly dialysis session duration (h)	12.2 ± 1.3	12.2 ± 1.2	0.38
Diabetes mellitus (%)	18.4	22	0.19
Past history of CVD (%)	41	47	0.23
BMI (kg/m^2)	25.6 ± 4.8	22.8 ± 3.9	0.03
Predialysis SBP (mmHg)	143 ± 23	145 ± 25	0.46
Predialysis DBP (mmHg)	76 ± 12	76 ± 13	0.49
Predialysis PP (mmHg)	67 ± 16	69 ± 17	0.37
Hemoglobin (g/100 ml)	10.9 ± 1.4	10.6 ± 1.4	0.19
PTH (pg/ml)	222 ± 202	258 ± 212	0.34
tHcy (μmol/l)	29.6 ± 7.8	25.9 ± 7	0.01
K_t/V	1.48 ± 0.30	1.50 ± 0.36	0.24
nPCR	1.05 ± 0.19	1.01 ± 0.25	0.11
CRP (mg/l)	4.6 ± 2.4	9 ± 4.8	<0.001
Serum albumin (g/l)	39.5 ± 2.8	34 ± 4.5	<0.001

BMI, body mass index; CIMS, chronic inflammation–malnutrition state; CRP, C-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; nPCR, protein catabolic rate; PP, pulse pressure; PTH, parathyroid hormone; SBP, systolic blood pressure; tHcy, total serum homocysteine level.

presence of inflammation–malnutrition was associated with lower body mass index and tHcy (Table 2).

Death

During a mean follow-up period of 54 months, 219 deaths (47.7%) occurred, 126 in patients with CIMS and 93 in patients without CIMS (114 (52%) of CVE, 65 in patients with CIMS, and 49 in those without CIMS). Survival was significantly lower in patients with CIMS (56 vs 40%; $P<0.001$).

Table 3 | Hazard ratio estimates for death derived from proportional-hazards modeling (multivariate adjusted model)

Variable	Hazard ratio	95% CI	*P*-value
CRP (mg/l)			
<4 (1st tertile)	1	—	—
[4; 8[(vs 1st tertile)	1.78	[1.17; 3.56]	0.02
$\geqslant 8$ (vs 1st tertile)	3.26	[1.78; 6.51]	0.002
Age (years)			
<62	1	—	—
[62; 72]	1.79	[0.96; 3.27]	0.07
$\geqslant 73$	3.33	[1.61; 5.72]	0.002
Serum albumin (g/l)			
<35 (1st tertile)	1	—	
[35; 39[(vs 1st tertile)	0.96	[0.86; 1.47]	0.27[2]
$\geqslant 39$ (vs 1st tertile)	0.81	[0.67; 0.91]	0.03
Diabetes mellitus			
No	1	—	—
Yes	1.68	[1.11–3.56]	0.01
History of CVE			
No	1	—	—
Yes	2.12	[1.37; 2.99]	0.006

CI, confidence interval; CRP, C-reactive protein; CVE, cadiovascular events.

tHcy levels were not different in patients who died during follow-up period in the overall cohort (29.1 ± 8 vs 28.2 ± 7.7 μmol/l; $P = 0.12$), but were higher in those without CIMS (31.5 ± 7.2 vs 26.4 ± 6.8 μmol/l; $P = 0.006$). Of note, in patients with CIMS, the association was in the opposite direction: lower levels of tHcy were observed in patients who died (24.6 ± 7.2 vs 29.1 ± 7.9; $P = 0.01$).

In the overall study population, univariate analysis revealed that age ($P<0.0001$), male gender ($P = 0.02$), a past history of CV disease ($P<0.0001$), smoking status ($P = 0.11$), diabetes mellitus ($P = 0.01$), pulse blood pressure (BP) ($P = 0.03$), C-reactive protein (CRP) ($P = 0.0001$), tHcy ($P = 0.12$), and low albumin concentration ($P = 0.008$) were associated with death.

Cox's regression analysis showed that age in the higher tertile (hazard ratio (HR), 3.33; 95% confidence interval (CI), 1.61–5.72) and previous history of CVE (HR, 2.12; 95% CI, 1.37–2.99) were risk factors for death. Diabetes mellitus also increased the risk of death (HR, 1.68; 95% CI, 1.11–3.56) as well as serum albumin concentration in the lowest tertile (Table 3). Patients in the two higher tertiles of serum CRP level showed an increased risk of death than those in the lowest tertile (Table 3).

tHcy was not associated with a significantly higher risk of death in the overall population (Table 4). In contrast, in the absence of CIMS, tHcy was positively associated with all-cause mortality. In the full model, the adjusted relative hazard of all-cause mortality associated with tHcy of greater than 31 μmol/l was 1.55 (95% CI, 1.12–4.72) (Table 4). However, in patients with CIMS, tHcy did not predict subsequent death after full adjustment (Table 4), mostly due to significant interaction between tHcy and CIMS parameters.

Table 4 | Hazard ratio estimates of tHcy for death derived from proportional-hazards modeling (multivariate adjusted model)

tHcy tertiles	Overall	Presence of CIMS (*n*=228)	Absence of CIMS (*n*=231)
T1 (low)	1	1	1
T2 (medium)	1.01 [0.76; 1.21]	0.91 [0.70; 1.03]	1.27 [0.97; 4.11]
T3 (high)	1.03 [0.82; 1.33]	0.78 [0.47; 1.02]	1.55 [1.12; 4.72]*

CIMS, chronic inflammation–malnutrition state; T, tertile.
*<0.05.

Table 5 | Hazard ratio estimates of tHcy for CV death derived from proportional hazards modeling (multivariate adjusted model)

tHcy tertiles	Overall	Presence of CIMS (*n*=228)	Absence of CIMS (*n*=231)
T1 (low)	1	1	1
T2 (medium)	1.16 [1.02; 1.35]*	0.95 [0.82; 1.08]	1.31 [1.00–3.11]
T3 (high)	1.51 [1.12; 1.72]*	0.90 [0.81; 1.01]	1.91 [1.23–3.23]*

CIMS, chronic inflammation–malnutrition state; T, tertile.
*<0.05.

In assessing cause-specific mortality, we observed an association between HHcy and CV mortality in the overall population. This relationship was found to be significant in patients without CIMS. In contrast, low tHcy concentrations conferred a trend towards an increased risk of CV-related mortality in patients with CIMS (Table 5).

DISCUSSION

Hemodialysis population presents a high rate of mortality compared to the general population. In our patients, the cumulative risk for death was found to be as high as 11% within 1 year. CV deaths accounts for more than one-half of deaths. A number of traditional and nontraditional risk factors have been identified to be of higher prevalence in this population potentially explaining this high rate of CV mortality.

Although interventional studies have remained contradictory,[13,14] a large majority of epidemiologic studies have reported a positive association between HHcy and CV disease in the general population. However, conflicting reports exist regarding the association between tHcy levels and mortality in patients on chronic dialysis. Although some authors have found that, similar to the general population, HHcy is associated with an increased risk of CVE and mortality in dialysis patients,[4–9] others have reported an inverse relationship in this population. The latter group found that dialysis patients with higher tHcy manifested a better survival rate than those with lower values. For instance, in a study on 117 hemodialysis patients, Suliman *et al.*[10] found that those patients with tHcy above the median (equal or greater than 24 μmol/l) showed a significantly lower 4-year survival rate than those with lower tHcy. However, more recently, Kalantar-Zadeh *et al.*[11] also reported that mortality rate was higher in the lowest tHcy quartile than in other quartiles. In this study, the lowest tHcy quartile contributed to a twofold increase in the risk of death. Wrone *et al.*[12] reported similar results in a prospective study on 510 chronic hemodialysis patients. Moreover, in this study, tHcy-lowering treatment did not reduce mortality maybe, at least in part, because of a small effect of treatment on tHcy concentrations. This paradoxical association puts under question the usefulness of treatment with folic acid in dialysis patients.

Our data do not support this finding. In our study, we demonstrate a strong, positive association of tHcy with overall and CV mortality in dialysis patients who do not present a chronic state of inflammation–malnutrition. In contrast, we observed a trend towards an inverse relationship between tHcy and all-cause and CV mortality in patients with CIMS. These results indicate that, similar to the general population, HHcy is a risk factor for all-cause and CV mortality in dialysis patients, and that this association is modified in individuals with CIMS. Consistent data indicate that tHcy might be considered a nutritional and/or inflammatory marker in dialysis patients.[10] tHcy is lower in patients with CIMS. A potential interaction between tHcy and these parameters is likely to hamper our ability to detect an independent role for HHcy on survival rate. tHcy results could not then be accurately interpreted without taking into consideration the presence or absence of CIMS.

The differences between results of our study and those found by other authors could be explained by a number of factors. Unlike most of other reports,[11,12] our study population did not suffer from a selection bias and included the whole dialysis population in two regions, Bourgogne and Franche-Comté. Whether the phenotype of our study population is different from that of other studies is difficult to assess, but exclusion of some of the patients in those studies might have changed or modulated their results. A bias of survival may eventually explain, at least in part, the paradoxical results of previous studies with the emerging concept of 'reverse epidemiology'. Incident patients with a higher number of risk factors did not survive long enough to be included in prospective studies or were excluded from the study because of a too short life expectancy.[11] Survival bias can potentially exert a strong influence on both epidemiologic and clinical studies, especially when patients with varying degrees of duration of dialysis are enrolled together. In our study, dialysis duration was forced into the model to persistently adjust our results for this confounding variable. We analyzed the role of tHcy with respect to numerous confounding factors including inflammatory (albumin, CRP) and nutritional (albumin, normalized protein catabolism ratio) parameters. In our opinion, considering the complex interactions existing between tHcy and CIMS, these factors cannot be analyzed separately. The difference in the frequency of CIMS is another factor to be considered. Compared to other studies, the proportion of patients with CIMS appears low in our trial.[15] The over-representation of this syndrome in other studies may have contributed to the association

found between low tHcy, a frequent feature of the CIMS, and poor survival. Our study has a relatively longer follow-up than the majority of studies reporting a 'reverse epidemiology' effect. CIMS is the main cause of early death after beginning the dialysis and short-term follow-up may have accentuated the survival bias and the consequences of the time–competition effect between risk factors, attenuating the impact of traditional risk factors.

Our study suggests that the impact of HHcy is dependent on the nutritional and inflammatory status. According to the studied population, HHcy may appear as a protective or deleterious factor. If a study population includes predominantly healthy dialysis patients and has a long-term follow-up, then the mortality–predictability of HHcy can be seen in the same way as it is in the general population. If a study population consists predominantly of malnourished and inflamed patients, then HHcy is a marker of better nutrition and is associated with better survival. Our study reconciles the advocates of both conventional and reverse epidemiology of tHcy in dialysis population. We showed clearly that none of both parties are wrong or right, but it is all about the confounding impact of malnutrition—inflammation, which can be so dominant to the extent that it even reverses such conventional associations. Our results also suggest that tHcy-lowering trials in dialysis patients should preferentially concern those without CIMS. Inclusion of patients with CIMS may compromise any chance to observe a significant impact of tHcy-lowering treatment in this population.

There are some limitations to our study. We studied the influence of a baseline tHcy and other factors on the occurrence of death in a population of prevalent dialysis patients. Serial measurements of these parameters in a longitudinal cohort design would certainly provide a more precise estimate of the risk in this population. Folate is one of the major determinant of homocysteine. Folate status was unknown and we cannot exclude both folate deficiency and folic acid supplementation in some patients. Also, some CV risk factors were not measured in our study, especially lipid parameters, which may have potentially influenced the results. Left ventricular hypertrophy was measured by electrocardiogram, which is a less sensitive measure than echocardiography. This may explain why left ventricular hypertrophy was associated neither with all-cause nor CV death. Finally, albumin is not only a nutritional marker but also reflects the inflammatory status. In our study, the presence of CIMS was defined as achievement of *a priori* cutoffs for any of the two variables, CRP and albumin. More accurate markers of malnutrition should probably be used to assess the respective role of inflammation and malnutrition. Nevertheless, the two entities are frequently associated.

CONCLUSION

Our study demonstrates that tHcy must be interpreted with caution in hemodialysis patients. Whereas low tHcy levels are associated with poor prognosis in patients with the CIMS, high tHcy levels independently predict death in patients without the CIMS. The concept of reverse epidemiology is probably only restricted to patients with the CIMS.

MATERIALS AND METHODS

Patients characteristics

Participants in the study were 459 chronic dialysis patients recruited from 10 dialysis centers of Bourgogne (Dijon, Macon, Chalon sur Saone, Auxerre, Sens) and Franche-Comté (Besançon, Montbéliard, Vesoul, Dole). All stable patients on chronic hemodialysis with dialysis duration of more than 3 months who showed no acute illnesses were included.

Confounding factors

Age, gender, weight, size, blood pressure, hemodialysis duration, weekly dialysis session duration, diabetes mellitus, smoking status, past history of CVE and different biological parameters were assessed upon inclusion.

Past history of CVE

A past history of CVE was defined by:

- *Coronary heart disease*: Myocardial infarction; coronary revascularization including coronary artery bypass surgery or percutaneous transluminal coronary angioplasty; typical history of angina with abnormal coronarography or myocardial scintigraphy.
- *Stroke/cerebrovascular disease*: Both hemorrhagic and non-hemorrhagic strokes; carotid endarterectomy.
- *Abdominal aortic or lower extremity arterial disease*: Abdominal aortic repair; lower extremity amputation; intermittent claudication confirmed by Doppler or arteriographic findings.

Nutritional status

Albumin concentration was determined. Body mass index was calculated (weight/(size)^2). Normalized protein catabolism ratio was calculated.[16]

To distinguish individuals with any evidence of CIMS, a composite variable combining serum albumin and CRP was used to categorize the study population into two subgroups. The presence of CIMS was defined as achievement of *a priori* cutoffs for any of the two variables from previously reported studies.[15] For serum albumin levels, the cutoff was less than 36 g/l, and for CRP, it was 10 mg/l or higher.

Smoking behavior

Subjects were categorized as current smokers or nonsmokers. A past history of smoking was also assessed.

Blood pressure

BP was measured using a semiautomatic device, based on an oscillometric method with the patients in a sitting position after having rested for more than 5 min. Mean (diastolic BP+ $\frac{1}{3}$(systolic BP − diastolic BP)) and pulse pressure (systolic BP−diastolic BP) were calculated.

Left ventricular hypertrophy

Left ventricular hypertrophy was defined by a Sokolow index equal to or greater than 35.

Residual renal function

Patients were categorized into two groups according to their daily urine output (less than 500 ml, equal to or greater than 500 ml).

Parathyroid hormone

A past history of parathyroidectomy was assessed through medical records. Intact parathyroid hormone (Elsa PTH, Scisbio, France) was measured using an immunoradiometric assay, with a normal range of 15–80 pg/ml.

Homocysteine

tHcy was measured using a previously described method.[17] Venous blood samples were drawn after an overnight fast. The blood sample was centrifuged for 15 min, and plasma was stored frozen at $-20^{\circ}C$. Hcy concentration, the sum of the acid-soluble (i.e. reduced Hcy, homocysteine, disulfide, and homocysteine–cysteine mixed disulfide) and protein-bound moieties were measured by high-performance liquid chromatography. This assay involves the following steps: reduction of the sample with tri-*n*-butylphosphine, precipitations of proteins, alkalinization of the supernatant with sodium borate, derivitization with 7-fluoro-2-oxa-1,3 diazole-4 sulfonate, followed by 8-aminonaphthalene-1,3,6-trisulfonic acid, and high-performance liquid chromatography separation with fluorescence detection. The normal values of plasma Hcy concentration ranged from 7 to 15 μmol/l. The precision of the assay corresponds to a coefficient of variation of less than 3%.

C-reactive protein

CRP was measured by Nephelometry (Beckman, Palo Alto, CA, USA).

Death

Death from all causes was ascertained by active follow-up through dialysis centers. The cause of death was considered to be CVE if it was due to coronary heart disease, stroke or complicated peripheral vascular disease. Sudden death was also counted in this group if a past history of CVE existed.

Two physicians independent of the study and without the knowledge of baseline characteristics were responsible for outcomes ascertainment. This analysis was performed without the knowledge of baseline characteristics.

Statistical analysis

Using log-rank tests on Kaplan–Meier nonparametric estimates of the survival without death distribution, we selected variables with a *P*-value lower than or equal to 0.20. All the variables depicted in Table 1 were analyzed. The selected variables were included into a Cox proportional-hazards model, and a backward stepwise selection process was performed, this time at a classical $\alpha = 0.05$. The time elapsed as dialysis initiation was found to vary between patients; therefore, this duration was forced into the Cox model as a covariate. Cox's proportional-hazards model was performed in the overall study population, and further age was split into tertiles (<64; 64–73; >73). Tobacco consumption was accounted for as currently smoking vs nonsmoking. Variables split into tertiles were replaced by dummy variables in the Cox model, which tested tertile 2 vs tertile 1 and tertile 3 vs tertile 1. Results are expressed as HR and 95% CI, with a *P*-value testing the null hypothesis: $HR = 1$. Therefore, when *P*-value is less than 0.05, HR is significantly different from 1, either greater than 1 (i.e. risk of death is increased) or less than 1 (i.e. risk of death is decreased). Assumptions of Cox models (log-linearity, proportionality of risk in time) were met in this analysis.

Comparisons of the distribution of categorical variables between patients with and without CIMS were performed by χ^2 tests, whereas continuous variables were compared using the nonparametric Wilcoxon's rank-sum test.

Analyses were performed on statview 5 (SAS institute Inc., Cary, NC, USA).

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