AGE-RELATED AND PROGNOSTIC RISK FACTORS IN DIALYSIS PATIENTS

Giovanni Luigi Tripepi

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AGE-RELATED AND PROGNOSTIC RISK FACTORS IN DIALYSIS PATIENTS

Leeftijdsgerelateerde en prognostische risicofactoren in dialysepatiënten

Proefschrift

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A Francesca, Margherita ed Emanuele.
Ai miei cari genitori, a mio fratello, Maria e Marco.
Al Prof. Zoccali e alla Dr.ssa Mallamaci per il grande
patrimonio di valori che mi hanno trasmesso.

All'Associazione IPNET per avermi supportato e sostenuto.

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Chapter 1

General Introduction

GENERAL INTRODUCTION

The replacement of renal function by dialysis is one of the major achievements of modern medicine. However, given the fact that renal failure shares common causes with cardiovascular diseases, dialysis patients are a population with a risk profile of almost unique severity (1). In fact, it was remarked that the risk of cardiovascular death of a young man (<30 years) on dialysis equals that of a healthy man over 85 years in the general population, suggesting that the pathogenetic processes linked to aging may be accelerated in dialysis patients.

The excess risk for cardiovascular morbidity and mortality in the dialysis population may be considered as the final, detrimental consequence of a process that starts very early in renal diseases (2). This thesis focuses on the end-stage phase of chronic renal failure, i.e. the stage in which only secondary and tertiary prevention measures can be undertaken. Since prevention and treatment strategies demand the identification of etiological and prognostic factors, well powered epidemiological studies in the setting of end stage renal disease (ESRD) represent an absolute public health priority. In this regard it is important noting that echocardiography provides fundamental information for risk stratification and risk monitoring in ESRD patients. Indeed, left ventricular (LV) mass and LV systolic function as measured by this technique are strong predictors of death and adverse cardiovascular outcomes in the dialysis population (3-4). On the other hand, echocardiography also allows detailed studies of atrial chambers. Although left atrium diameter or volume measurements have occasionally been used to monitor changes in circulating volume in ESRD, to our knowledge there are no large scale, systematic studies in ESRD patients aimed at defining 1) the prognostic value of single and repeated measurements of left atrial volume in the dialysis population and 2) the predictive power of specific biomarkers of left atrial enlargement in ESRD patients. Furthermore, while traditional risk factors dominate the scene in the general population, non traditional risk factors like inflammation (high C-Reactive Protein, CRP) (5-6), high circulating levels of atrial and brain natriuretic peptides (ANP and BNP, an expression of LV hypertrophy and LV systolic dysfunction) (7-8), enhanced levels of norepinephrine (a marker of sympathetic activity) (9-10), hyperparathyrodism (11) and accumulation of the endogenous inhibitor of the nitric oxide (NO) synthase, asymmetric dimethyl-arginine (ADMA) (12), are all markers of high cardiovascular risk of ESRD patients. To obtain a quantitative insight on the etiological and prognostic role of non traditional risk factors in ESRD patients, we performed a detailed statistical analysis in the cardiovascular risk extended evaluation in dialysis (CREED) patients database including an incident-prevalent cohort of ESRD patients. Since aging per se and/or by increasing the time of exposure to other risk factors triggers important effects on cardiovascular system, we always considered age as etiological/prognostic factor in multivariate modelling and specifically investigated the relationship between aging and alterations in LV mass and LV systolic dysfunction in the dialysis population. The issue is of importance

because cardiomyopathy in ESRD may be in part due to a specific cluster of risk factors linked to senescence.

The first part of the thesis (chapter 2) focuses on the design of the Cardiovascular Risk Extended Evaluation in Dialysis patients (CREED) study that represents the source database for the analyses described in this thesis. The second part (chapter 3) deals with aging, functional and metabolic risk markers in end stage renal diseases. The studies described in chapter 4 concern risk factors of mortality and cardiovascular events in the dialysis population. In the general discussion (chapter 5), the main findings of this thesis are dicussed.

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Chapter 2

Design of the Cardiovascular Risk Extended Evaluation in Dialysis patients (CREED) study

DESIGN OF THE CARDIOVASCULAR RISK EXTENDED EVALUATION IN DIALYSIS PATIENTS (CREED) STUDY

The **C**ardiovascular **R**isk **E**xtended **E**valuation in **D**ialysis patients (**CREED**) study is a prospective study including an incident-prevalent cohort of about 300 patients on chronic dialysis treated in two urban areas of the Southern of Italy (Reggio Calabria and Catania). The CREED study started about 14 years ago (1997) and it was funded by Regione Calabria, Department of Health. It was conceived to investigate the relationship between traditional and non traditional cardiovascular risk factors, carotid atherosclerosis and alterations in left ventricular mass, geometry and function as well as to analyse the prognostic value of specific and new biomarkers for predicting/explaining the incidence rate of mortality and fatal and non cardiovascular events in the dialysis population. Among 300 patients enrolled, the echocardiographic measurements were available in 254 cases (85%) and the eco-color Doppler studies of the carotid arteries in 134 cases (45%). To date, the follow-up duration is about 14 years and the prospective data collection is still ongoing. The study was carried out in collaborations with local, national and international research institutions.

Beyond its prospective nature, the winning card of the CREED study was the creation of a large serum and plasma biobank that allowed us to measure new and unsuspected risk factors in patients with end stage renale diseases (ESRD) as well as the collection of information, by echocardiography and ECO-color doppler, on heart and vascular status of enrolled patients.

The CREED database contains about 250 variables including traditional (Framingham) risk factors, factors peculiar to ESRD (like calcium, phosphate, haemoglobin and duration of dialysis treatment), information on medications and on background cardiovascular complications, echocardiographic and eco-color Doppler data, biomarkers of inflammation/infection, malnutrition and endothelial dysfunction. The study was conceived by Prof. Carmine Zoccali (responsible of the study) and the data collection and the clinical supervision was performed by Dr Francesca Mallamaci (as the outcome assessor).

The main limitation of the CREED study is that it did not schedule specific interventions in order to confirm the causal role of all risk factors identified.

Chapter 3

Aging, functional and metabolic risk markers in end stage renal disease (ESRD)

Chapter 3.1

Biomarkers of Left Atrial Volume: A Longitudinal Study in Patients with End Stage Renal Disease (ESRD)

ABSTRACT

Left atrial volume (LAV) has recently emerged as an useful biomarker for risk stratification and risk monitoring in patients with end stage renal disease (ESRD). We investigated the relationship between cardiac natriuretic peptides (ANP and BNP) and norepinephrine (NE) with LAV and LAV changes over time in 199 ESRD patients.

At baseline, LAV was directly related to BNP (r=0.60), ANP (r=0.59) and NE (r=0.28) (P<0.001) and these relationships held true in multiple regression models adjusting for potential confounders (P≤0.003). In the longitudinal study (17±2 months) LAV increased from 9.8±4.6 ml/$m^{2.7}$ to 10.9±5.4 ml/$m^{2.7}$ (+11%). In a multiple linear regression model, BNP (β=0.28, P=0.003), ANP (β=0.22, P=0.03) and NE (β=0.27, P=0.003) predicted LAV changes. The area under the ROC curve (AUC) for predicting LAV changes (>3 ml/$m^{2.7}$/yr) of a risk score based on standard risk factors was 0.72. Plasma BNP (+12%, P=0.004), ANP (+8%, P=0.03), NE (+8%, P=0.05) and midwall fraction shortening (+8%, P=0.05) increased to a significant extent the AUC while LV mass did not (+5%, P=0.18). Predictive models including BNP, ANP and NE maintained a satisfactory discriminatory power for LAV and LAV changes also when tested by a bootstrap re-sampling technique

BNP and ANP are strongly related to LAV in the ESRD patients and predict LAV changes over time in these patients. Because an increased LAV underlies diastolic dysfunction and/or volume overload, i.e. potentially modifiable risk factors, the measurement of the plasma concentration of these compounds might be useful for risk stratification and for guiding treatment in dialysis patients.

Key words: atrial natriuretic peptide, brain natriuretic peptide, end stage renal disease, left atrial volume, norepinephrine.

INTRODUCTION

Left atrial volume (LAV) as measured by echocardiography has recently emerged as a biomarker of potential value for risk stratification (1) and risk monitoring (2) in patients with end stage renal disease (ESRD). Indeed, LAV and LAV changes over time predict death and cardiovascular outcomes also beyond established echocardiographic markers of high cardiovascular risk like left ventricular mass (LVM) and LV systolic function (1-2). Even though echocardiography is formally recommended as a fundamental tool for risk stratification in ESRD patients (3), because of cost and logistic problems, this technique is applied less than needed in many centres. Biomarker research on anatomic and functional alterations of the heart is a growing, promising clinical research area (4). We have previously shown that atrial natriuretic peptide (ANP), a hormone mainly produced in the atrium, and brain natriuretic peptide (BNP), a hormone secreted by ventricular cardio-myocytes, are reasonably accurate markers of LVM and LV systolic function in ESRD patients (5-6). The relationship between cardiac natriuretic peptides and LAV and the diagnostic and prognostic value of these peptides for LAV enlargement and for LAV evolution over time have never been tested in well powered longitudinal studies in ESRD population. The issue is of importance because factors predisposing to LAV enlargement in ESRD are in part modifiable by pharmacological and non pharmacological interventions.

High plasma norepinephrine (NE) is a risk factor for concentric left ventricular hypertrophy (LVH) in ESRD (7), which is in turn a strong determinant of altered diastolic dysfunction (8). Because, independently of other risk factors, altered LV diastolic function is associated with LAV enlargement (1), we hypothesised that high plasma NE may contribute to explain the variability in LAV and LAV changes over time in ESRD.

In the present study, we have investigated whether plasma levels of cardiac natriuretic peptides and NE are associated with LAV and whether on longitudinal observation these biomarkers predict LAV progression in a cohort of ESRD patients without clinical evidence of heart failure at baseline.

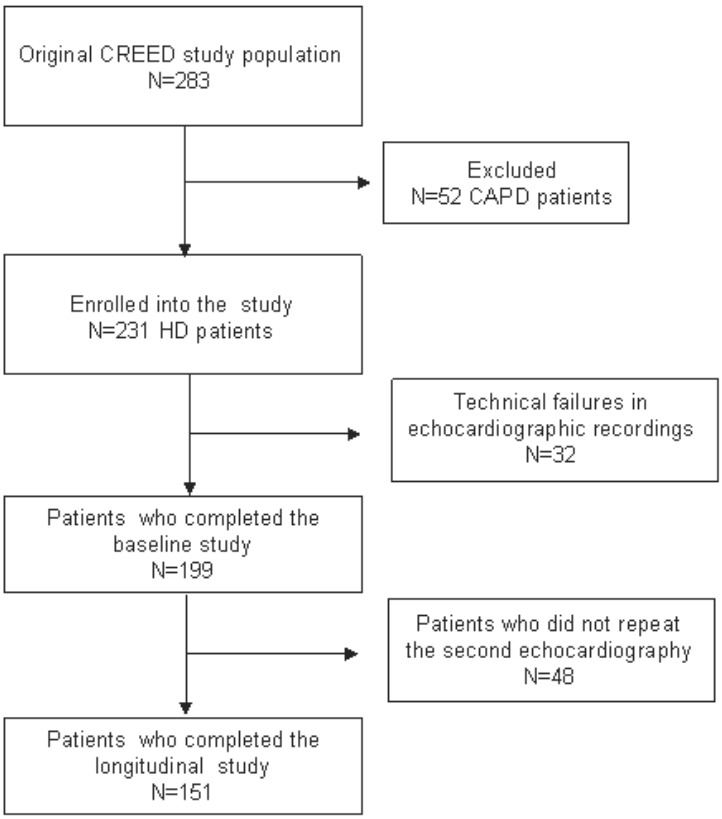
METHODS

The protocol was in conformity to the Declaration of Helsinki and informed consent was obtained from each participant. All studies were performed between 8 A.M. and 1 P.M.

Study cohort

The original **C**ardiovascular **R**isk **E**xtended **E**valuation in **D**ialysis patients (**CREED**) study cohort was formed by 283 ESRD patients [231 on haemodialysis (HD) and 52 on chronic ambulatory peritoneal dialysis (CAPD)](Fig. 1). By protocol, we excluded 52 patients because

Figure 1. Enrolment scheme of the study.



Abbreviations: CREED=cardiovascular risk extended evaluation in dialysis patients; **CAPD**=chronic ambulatory peritoneal dialysis; **HD**= hemodialysis.

on CAPD. Among the remaining 231 HD patients, 32 patients were excluded for technical failure in echocardiographic recordings. Hence, 199 HD patients (age 59±15 years, 111 Males and 88 Females) were available for the baseline analysis (Table 1). These patients had been on regular dialysis treatment (RDT) for at least 6 months (median dialysis vintage: 43 months, inter-quartile range 20-110 months). The enrolment criteria in this cohort were no history of congestive heart failure [defined as dyspnea in addition to two of the following conditions - raised jugular pressure, bibasilar crackles, pulmonary venous hypertension or interstitial edema on chest X ray, requiring hospitalization or extra ultra-ultrafiltration], LV ejection fraction >35% and no inter-current or terminal illnesses. The average Kt/V in these patients was 1.22±0.27. One hundred and nine patients were on treatment with erythropoietin. Seventy-seven patients were being treated with anti-hypertensive drugs (53 on mono-therapy with ACE inhibitors, AT-1 antagonists, calcium channel blockers, alpha and beta-blockers and 24 on double or triple therapy with various combinations of these drugs). All patients were being treated thrice weekly with standard bicarbonate dialysis (Na 138 mMol/L, HCO3 35

Table 1 Main demographic, somatometric, clinical and biochemical data in the study population.

		Patient who repeated the echocardiographic study (n=151)		
	Original study population (n=199)	**First visit**	**Second visit**	**P (1st visit vs 2nd visit)**
Age (years)	59±15	57.9±1.2	59.4±15.3	<0.001
Male sex n. (%)	111(56%)	85(56%)	85(56%)	1.0
Smokers n. (%)	78(39%)	53(35%)	53(35%)	1.0
Diabetics n. (%)	26(13%)	17(11%)	17(11%)	1.0
On anti-hypertensive therapy n. (%)	77(39%)	54(36%)	50(33%)	0.72
Systolic pressure (mmHg)	140±25	139±24	136±27	0.13
Diastolic pressure (mmHg)	77±13	76±13	75±14	0.15
Inter-dialysis weight gain (%)	4.2±1.2	4.3±1.2	4.5±1.7	0.27
Kt/V	1.22±0.27	1.23±0.27	1.33±0.25	<0.001
Cholesterol (mg/dL)	206±56	203±55	175±47	<0.001
Hemoglobin (g/L)	106±19	105±18	109±16	0.004
Albumin (g/L)	42±5	42±5	36±5	<0.001
Calcium*Phosphate ($mMol^2/L^2$)	4.5±1.2	4.5±1.2	4.3±1.2	0.006
C-Reactive Protein (mg/L)	7.6(3.4-16.3)	7.4(3.4-16.0)	NA	…
Homocysteine (µMol/L)	26.5(19.3-42.7)	24.3(18.2-38.3)	NA	…
Norepinephrine (nMol/L)	3.13(1.78-5.65)	3.26(1.71-5.73)	NA	…
Brain natriuretic peptide (pMol/L)	22.5(8.4-43.4)	22.3(8.4-37.6)	NA	…
Atrial natriuretic peptide (pMol/L)	22.7(14.9-38.8)	22.1(14.9-38.0)	NA	…
Left atrial volume ($ml/m^{2.7}$)	10.2±5.0	9.8±4.6	10.9±5.4	<0.001
Left ventricular mass index ($g/m^{2.7}$)	61.3±18.7	59.9±17.9	64.0±19.4	<0.001
Midwall fractional shortening (%)	14.5±3.3	14.7±3.3	14.4±2.7	0.19
E/A ratio	0.79±0.28	0.80±0.30	0.79±0.28	0.55

Data are expressed as mean ± SD, median and inter-quartile range or as percent frequency, as appropriate. The P value in the last column refers to the comparison between variables in the first visit and the same set of variables in the second visit.
KT/V denotes fractional urea clearance.

mMol/L, K 1.5 mMol/L, Ca 1.25 mMol/L, Mg 0.75 mMol/L) and cuprophan or semi-synthetic membranes (dialysis filters surface area: 1.1-1.7 m^2).

Patients who repeated the echocardiographic study

Forty-eight patients out of 199 who entered into the study did not repeat the second echocardiography because they died, were transplanted or for logistic reasons (Fig. 1). Thus, 151 ESRD patients underwent a first and a second echocardiography after a mean follow-up of

17±2 months. No patient had severe valvular heart disease and only a minority had mild to moderate valvular heart disease (n=16).

Echocardiography

These studies were performed mid-week in a non-dialysis day. At the time of the echocardiographic examination, investigators involved in echocardiographic studies were unaware of patients' clinical data. Left ventricular mass (LVM) was calculated according to the Devereux formula and indexed to height$^{2.7}$ (LVMI), as proposed by De Simone (9). Left atrial volume (LAV) was calculated by the biplane method of discs (10) at the end of left ventricle systole. LAV data were analyzed as height$^{2.7}$ indexed estimates because this indexation provides the best prognostic power in dialysis patients (1). Mid-wall fractional shortening (mwFS) was calculated according to the method of Shimizu et al (11) as described in full detail by De Simone (12). Changes in LAV were quantified by subtracting LAV at the second study from that obtained at baseline study and by factoring this difference for the time interval between the two studies. As normal value for LAV we considered the 95th percentile of the LAV distribution in a group of 100 subjects without heart disease (that is 12 ml/m$^{2.7}$) (1).

Blood pressure and inter-dialysis weight gain measurements

Pre-dialysis blood pressure (13) and inter-dialysis weight gain were calculated as average values of 12 recordings (3/week) taken by the nurses during the month preceding the echocardiographic study.

Biochemical Measurements

Blood sampling for biochemical measurements were performed before echocardiographic studies. Serum lipids, albumin, calcium, phosphate and haemoglobin were measured by standard methods in the routine clinical laboratory. The plasma concentration of norepinephrine (NE) was measured by a commercially available RIA kit (Amicyl-test TM, Immunological Laboratories, Hamburg, Germany). The upper limit of the normal range of plasma nor-epinephrine in our laboratory is 3.54 nMol/L. The plasma concentrations of α-human atrial natriuretic peptide (ANP) (normal value <27 pMol/L) and brain natriuretic peptide (BNP) (normal value <7.8 pMol/L) were measured by commercially available RIA kits (Peninsula Laboratory Europe Ltd, St. Helens, Merseyside, UK) after pre-extraction by reverse chromatography (Seppak C-18 Cartridges, Waters, Mildford MA). Recovery was >80% both for ANP and BNP. There was no cross reactivity between the two assays. The between-assay and within-assay coefficients of variability were 8% and 10% for ANP and 9% and 11% for BNP. The methods used for the determination of serum C-Reactive Protein (CRP) and plasma total homocysteine were detailed in a previous study (14).

Statistical Analysis

Data are expressed as mean ±± SD (normally distributed data), median and inter-quartile range (non normally distributed data) or as percent frequencies. Within subjects comparisons were made by paired t-test and Chi Squared test, as appropriate. The relationship between paired variables was analyzed by Pearson product moment correlation coefficient. Variables which showed a positively skewed distribution were log transformed (lg_{10}) before the correlation study.

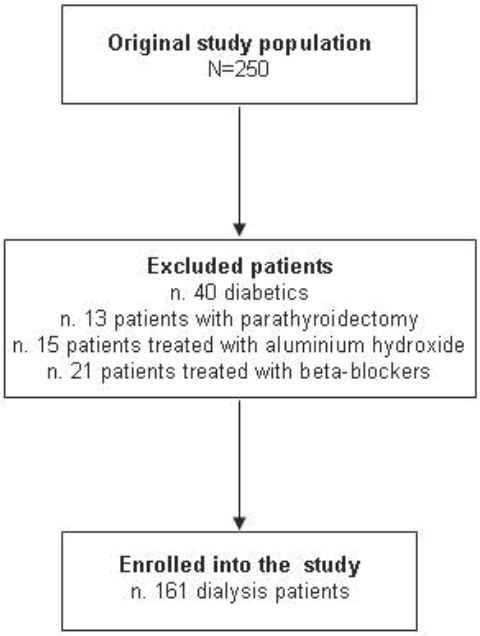
The independent correlates of baseline LAV and LAV changes were identified by univariate and multiple linear regression analyses. The following variables were considered for multiple linear regression analyses: plasma ANP, plasma BNP and plasma NE as well as age, sex, smoking, diabetes mellitus, systolic pressure, LV mass (LVMI), LV systolic function (mwFS), total cholesterol, haemoglobin, albumin, calcium phosphate product, homocysteine, CRP, CV comorbidities and inter-dialysis weight gain. Data are expressed as regression coefficients, standard errors (SE) of regression coefficients and standardized regression coefficient (β) and P value. The predictive value of ANP, BNP and NE for both LAV enlargement at baseline (> 12 $ml/m^{2.7}$) and LAV enlargement over time [> 3 ml/m2.7/year which corresponds to the average + 1 standard deviation of LAV changes in the whole study cohort) beyond and above that provided by standard risk factors was also investigated by analysing the area under Receiving Operating Curves (Area Under the Curve, AUC). ROC curves were compared by using the standard method (15). To assess the discriminatory power of prediction models, a bootstrap re-sampling technique of 200 samples was performed (16). Statistical analyses were done with standard statistical packages (SPSS for Windows Version 9.0.1, Chicago, Illinois, USA and R for Windows Version 2.8.1 by R Foundation for Statistical Computing).

RESULTS

Initial survey

The original study population included 199 haemodialysis patients (Table 1). Plasma ANP and BNP exceed the upper limit of the corresponding normal range in 149 (76%) and in 82 (42%) ESRD patients, respectively. Plasma NE was higher than the normal threshold in 91 patients (46%). On univariate analysis, plasma BNP ($r=0.60$, $P<0.001$), plasma ANP ($r=0.59$, $P<0.001$) as well as plasma NE ($r=0.28$, $P<0.001$) were all significantly related to LAV (Fig. 2). Of note, the association between BNP and ANP with LAV (Fig. 2) was coherently stronger than that between the same peptides with LVMI (BNP-LVMI: $r=0.53$, $P<0.001$; ANP-LVMI: $r=0.53$, $P<0.001$) and mwFS (BNP-mwFS: $r=-0.52$, $P<0.001$; ANP-mwFS: $r=-0.42$, $P<0.001$). The relationship between natriuretic peptides and NE with LAV was almost unmodified by multiple data adjustment (Table 2) controlling for Framingham risk factors, factors peculiar to ESRD, homocysteine, CRP, CV comorbidities and inter dialysis weight gain (a surrogate of volume

Figure 2. Relationship between plasma BNP, plasma ANP and plasma Norepinephrine with baseline LAV.



Data are Pearson product moment correlation coefficient and P value. Since BNP, ANP and NE had a positively skewed distribution these variables were log transformed (lg_{10}) before the correlation study. **Abbreviations: ANP**=atrial natriuretic peptide; **BNP**=brain natriuretic peptide; **LAV**=left atrial volume; **NE**= norepinephrine.

Table 2 Multiple regression models of baseline LAV

a) BNP based model	Units of measure	b ± SE	β (P)
BNP	**lg_{10}pMol/L**	**5.50±0.67**	**0.56(<0.001)**
CV comorbidities	**0=no; 1=yes**	**1.71±0.67**	**0.17(0.01)**
Haemoglobin	g/L	-0.02±0.02	-0.07(0.28)
Homocysteine	lg_{10}µMol/L	1.00±1.20	0.05(0.41)
C-Reactive Protein	lg_{10}mg/L	-0.56±0.73	-0.05(0.44)
Albumin	g/L	0.05±0.07	0.05(0.49)
Age	years	0.02±0.02	0.05(0.50)
Cholesterol	mg/dL	0.003±0.006	-0.04(0.57)
Sex	0=F; 1=M	-0.12±0.71	-0.01(0.87)
Inter-dialysis weight gain	kg	-0.05±0.40	-0.008(0.90)
Systolic pressure	mmHg	-0.001±0.01	-0.007(0.91)
Calcium phosphate product	$mMol^2/L^2$	-0.03±0.26	-0.007(0.91)
Diabetes	0=no; 1=yes	-0.04±0.93	-0.003(0.97)
Smoking	0=no; 1=yes	-0.02±0.69	0.002(0.97)

Constant:-1.477

b) ANP based model	Units of measure	b ± SE	β (P)
ANP	**lg_{10}pMol/L**	**8.89±1.09**	**0.57(<0.001)**
CV comorbidities	**0=no; 1=yes**	**1.79±0.67**	**0.18(0.008)**
C-Reactive Protein	lg_{10}mg/L	-0.84±0.73	-0.07(0.25)
Haemoglobin	g/L	-0.01±0.02	-0.05(0.42)
Albumin	g/L	0.06±0.07	0.06(0.44)
Homocysteine	lg_{10}µMol/L	0.79±1.21	0.04(0.51)
Sex	0=F; 1=M	-0.40±0.72	-0.04(0.58)
Age	years	0.007±0.02	0.02(0.76)
Systolic pressure	mmHg	0.0003±0.01	0.02(0.80)
Inter-dialysis weight gain	kg	0.09±0.40	0.01(0.83)
Calcium phosphate product	$mMol^2/L^2$	-0.05±0.26	-0.02(0.84)
Smoking	0=no; 1=yes	0.03±0.70	0.003(0.96)
Cholesterol	mg/dL	0.00004±0.006	0.00(1.00)
Diabetes	0=no; 1=yes	0.004±0.93	0.00(1.00)

Constant:-9.452

c) Norepinephrine based model	Units of measure	b ± SE	β (P)
Norepinephrine	**lg_{10}nMol/L**	**1.89±0.62**	**0.31(0.003)**
CV comorbidities	**0=no; 1=yes**	**-0.62±0.44**	**0.24(0.002)**
Systolic pressure	**mmHg**	**0.007±0.009**	**0.15(0.04)**
Albumin	**g/L**	**-0.06±0.04**	**-0.15(0.05)**
Homocysteine	lg_{10}µMol/L	1.61±0.78	0.07(0.33)
Haemoglobin	g/L	-0.004±0.01	-0.12(0.10)
Smoking	0=no; 1=yes	0.43±0.47	0.11(0.14)
Inter-dialysis weight gain	Kg	0.41±0.28	-0.09(0.24)
Sex	0=F; 1=M	-0.19±0.48	-0.04(0.59)
C-Reactive Protein	lg_{10}mg/L	0.17±0.48	-0.04(0.62)
Calcium phosphate product	$mMol^2/L^2$	0.14±0.17	-0.03(0.66)
Diabetes	0=no; 1=yes	0.036±0.70	0.03(0.66)
Cholesterol	mg/dL	0.002±0.004	-0.02(0.75)
Age	years	-0.01±0.01	-0.01(0.85)

Constant:13.194

Data are expressed as regression coefficients (b), standard error (SE) of the regression coefficient, standardized regression coefficients (β) and P values.

Abbreviations: CV=cardiovascular; **ANP**=atrial natriuretic peptide; **BNP**=brain natriuretic peptide.

expansion). Further adjustment for LVMI and mwFS produced only a moderate reduction in the strength of these associations which remained all highly significant (BNP-LAV: β=0.39, P<0.001; ANP-LAV: β=0.41, P<0.001; NE-LAV: β=0.20, P=0.001).

Fifty-five patients out of 199 (28%) had LAV above the upper limit of the normal range (cut-off: 12 ml/$m^{2.7}$). On ROC curve analysis both BNP and ANP had higher discriminatory power for identifying patients with baseline LAV >12 ml/$m^{2.7}$ than plasma NE, LVMI and mwFS (Table 3a) and both these peptides added significant discriminatory power to a score for LAV enlargement based on standard risk factors (Model 1) (Table 3b) while the gain in predictive value provided by LVMI, mwFS and NE was smaller and not significant (P≥0.07) (Table 3b). Neither the simultaneous inclusion of LVMI and mwFS into the risk score nor combinations

Table 3 ROC curve analyses of natriuretic peptides, norepinephrine, left ventricular mass index and midwall fractional shortening for LAV enlargement (a) and additional discriminatory power of these variables beyond and above that provided by standard risk factors (basic model) (b). LAV enlargement at baseline was defined as a LAV exceeding the upper limit of the normal range (> 12 ml/$m^{2.7}$) in a series of 100 individuals without cardiovascular disease studied at our institution (1). LAV enlargement progression over time was defined as an increase exceeding the average change +1 standard deviation (3 ml/$m^{2.7}$/yr).

a)

	AUC for LAV > 12 ml/$m^{2.7}$		**AUCs for LAV changes > 3 ml/$m^{2.7}$/yr**	
	Original set	Bootstrap validation	Original set	Bootstrap validation
ANP	0.82	0.83	0.68	0.70
BNP	0.83	0.82	0.75	0.75
NE	0.65	0.72	0.70	0.72
BNP+NE	0.81	0.86	0.73	0.72
BNP+ANP	0.82	0.78	0.75	0.67
ANP+NE	0.81	0.82	0.72	0.63
LVMI	0.77	0.77	0.61	0.59
mwFS	0.75	0.73	0.71	0.71
LVMI + mwFS	0.79	0.79	0.71	0.71

b)

	AUC for LAV > 12 ml/$m^{2.7}$		**AUCs for LAV changes > 3 ml/$m^{2.7}$/yr**	
	Original set	Bootstrap validation	Original set	Bootstrap validation
***Model 1**	0.73	0.76	0.72	0.60
Model 1+ ANP	0.86	0.85	0.80	0.78
Model 1+ BNP	0.85	0.83	0.84	0.78
Model 1+ NE	0.80	0.79	0.80	0.81
Model 1+ BNP+NE	0.86	0.87	0.87	0.86
Model 1+ BNP+ANP	0.87	0.89	0.84	0.82
Model 1+ ANP+NE	0.87	0.89	0.84	0.80
Model 1+ LVMI	0.80	0.79	0.77	0.64
Model 1+ mwFS	0.81	0.81	0.80	0.63
Model 1+ LVMI + mwFS	0.83	0.83	0.81	0.64

*__Model 1__: includes: age, sex, smoking, diabetes, cholesterol, CV comorbidities, haemoglobin, albumin, calcium phosphate product, C-Reactive Protein, homocysteine and inter-dialysis weight gain.
Abbreviations: CV=cardiovascular; **ANP**=atrial natriuretic peptide; **BNP**=brain natriuretic peptide; **LVMI**=left ventricular mass index; **mwFS**= midwall fractional shortening.

of ANP, BNP and NE measurements did provide significant additional discriminatory power in comparison to that by BNP or ANP considered separately. Bootstrap re-sampling validation substantially confirmed these results (Table 3 a-b).

Longitudinal study

Patients who repeated echocardiography (n=151) did not differ from those of the original cohort (n=199) (Table 1). LAV increased from 9.8±4.6 ml/$m^{2.7}$ to 10.9±5.4 ml/$m^{2.7}$ (+11%) (P<0.001). Individual changes in LAV were inversely related to baseline LAV (r=-0.17, P=0.04) implying that regression to the mean may be a confounder for the interpretation of the evolution of LAV over time. To allow for this relationship we adjusted LAV changes for corresponding baseline values and used this estimate as an outcome measure in the data analysis. Significant differences between the first and the second echocardiographic study were also observed in serum cholesterol, Kt/V, haemoglobin, albumin and calcium phosphate product (Table 1). The rate of increase in LAV was higher in patients with concentric LVH (1.20±2.70 ml/$m^{2.7}$/yr) than in those without (0.52±2.07 ml/$m^{2.7}$/yr). Plasma BNP (r=0.23, P=0.004), ANP (r=0.17, P=0.04), NE (r=0.25, P=0.002), homocysteine (r=0.19, P=0.02), mwFS (r=-0.20, P=0.01) and inter-dialysis weight gain (r=0.16, P=0.05) were all related to LAV changes. Multiple linear regression analysis indicated that the association between plasma BNP (β=0.28, P=0.003), ANP (β=0.22, P=0.03) and NE (β=0.27, P=0.003) with LAV changes (Table 4) was independent of other risk factors.

The predictive value of BNP, ANP and NE for changes in LAV was further investigated by ROC curve analysis. Patients were divided into two groups: below and above our pre-specified definition of progressive LAV enlargement over time [i.e. a LAV increase exceeding the average change + 1SD (3 ml/$m^{2.7}$/yr)]. This analysis showed that BNP had a higher discriminatory power (AUC=0.75) as compared to that of ANP, NE, LVMI and mwFS (Table 3a). The area under

Table 4 Multiple regression models for LAV changes (adjusted for LAV at baseline).

a) BNP based model	Units of measure	b ± SE	β (P)
BNP	**lg_{10} pMol/L**	**1.32±0.43**	**0.28(0.003)**
Homocysteine	**lg_{10} µMol/L**	**1.68±0.78**	**0.20(0.03)**
Inter-dialysis weight gain	**kg**	**0.60±0.27**	**0.20(0.03)**
CV comorbidities	0=no; 1=yes	-0.71±0.44	-0.15(0.11)
Calcium phosphate product	$mMol^2/L^2$	0.20±0.17	0.10(0.24)
Cholesterol	mg/dL	0.0004±0.004	0.07(0.44)
Albumin	g/L	-0.02±0.04	-0.05(0.62)
Diabetes	0=no; 1=yes	-0.28±0.68	-0.04(0.68)
Systolic pressure	mmHg	-0.002±0.009	-0.02(0.80)
Sex	0=F; 1=M	0.12±0.46	0.03(0.80)
Age	years	-0.003±0.01	-0.02(0.82)
C-Reactive Protein	lg_{10} mg/L	0.09±0.48	0.02(0.84)
Haemoglobin	g/L	0.0009±0.01	0.007(0.94)
Smoking	0=no; 1=yes	-0.02±0.47	-0.004(0.96)

Constant:-5.055

b) ANP based model	Units of measure	b ± SE	β (P)
ANP	**lg_{10} pMol/L**	**1.60±0.71**	**0.22(0.03)**
Inter-dialysis weight gain	**kg**	**0.60±0.28**	**0.20(0.03)**
Homocysteine	**lg_{10} µMol/L**	**1.60±0.80**	**0.19(0.05)**
CV comorbidities	0=no; 1=yes	-0.69±0.45	-0.14(0.12)
Calcium phosphate product	$mMol^2/L^2$	0.19±0.17	0.10(0.25)
Cholesterol	mg/dL	0.004±0.004	0.09(0.35)
Albumin	g/L	-0.03±0.04	-0.07(0.52)
Diabetes	0=no; 1=yes	-0.29±0.70	-0.04(0.68)
Age	years	-0.005±0.01	-0.04(0.71)
Sex	0=F; 1=M	0.09±0.47	0.02(0.85)
Haemoglobin	g/L	0.001±0.01	0.01(0.91)
Systolic pressure	mmHg	0.0003±0.009	0.003(0.97)
C-Reactive Protein	lg_{10} mg/L	0.02±0.49	0.003(0.97)
Smoking	0=no; 1=yes	0.01±0.47	0.002(0.98)

Constant:-5.462

c) Norepinephrine based model	Units of measure	b ± SE	β (P)
Norepinephrine	**lg_{10} nMol/L**	**1.89±0.62**	**0.27(0.003)**
Homocysteine	**lg_{10} µMol/L**	**1.61±0.78**	**0.19(0.04)**
Inter-dialysis weight gain	Kg	0.41±0.28	0.14(0.15)
Albumin	g/L	-0.06±0.04	-0.13(0.15)
CV comorbidities	0=no; 1=yes	-0.62±0.44	-0.13(0.16)
Age	years	-0.01±0.01	-0.10(0.31)
Smoking	0=no; 1=yes	0.43±0.47	0.09(0.36)
Calcium phosphate product	$mMol^2/L^2$	0.14±0.17	0.07(0.40)
Systolic pressure	mmHg	0.007±0.009	0.08(0.40)
Cholesterol	mg/dL	0.002±0.004	0.04(0.66)
Sex	0=F; 1=M	-0.19±0.48	-0.04(0.69)
Haemoglobin	g/L	-0.004±0.01	-0.03(0.71)
C-Reactive Protein	lg_{10} mg/L	0.17±0.48	0.03(0.72)
Diabetes	0=no; 1=yes	0.036±0.70	0.005(0.96)

Constant:-1.743

Data are expressed as regression coefficients (b), standard error (SE) of the regression coefficient, standardized regression coefficients (β) and P values.

Abbreviations: CV=cardiovascular; **ANP**=atrial natriuretic peptide; **BNP**=brain natriuretic peptide.

the ROC curve of a risk score based on Framingham risk factors, CV comorbidities, haemoglobin, albumin, calcium phosphate product, CRP, homocysteine and inter-dialysis weight gain was 0.72. Plasma BNP (+12%, P=0.004), ANP (+8%, P=0.03), NE (+8% gain, P=0.05) and mwFS (+8% gain, P=0.05) increased to a significant extent the area under the ROC curve while LVMI did not (+5%, P=0.18) (Table 3b). Neither the simultaneous inclusion of LVMI and mwFS nor combinations of ANP, BNP and NE did provide significant additional discriminatory information in comparison to the risk score and BNP.

The discriminatory power of the model including BNP and NE (area under ROC curve: 87%) was marginally superior to that including the two cardiac natriuretic peptides (area under

ROC curve: 84%) or to that including ANP and NE (area under ROC curve: 84%). Natriuretic peptides and NE maintained an adequate discriminatory power for identifying LAV enlargement also when tested by a bootstrap re-sampling technique (Table 3 a-b).

DISCUSSION

In a cohort of ESRD patients without clinical evidence of heart failure, cardiac natriuretic peptides and NE emerged as independent correlates of LAV and predicted LAV enlargement over time independently of Framingham risk factors, factors peculiar to ESRD and other risk factors. Notably, both in the initial survey and in the longitudinal analysis the predictive power for LAV enlargement of these biomarkers was higher than that provided by LV mass and function.

Left atrium in ESRD patients

LA enlargement as measured by echocardiography is a common finding in the dialysis population (1). LV systolic and diastolic dysfunction (1,17), valvular heart disease (18), extracellular volume expansion (19-21), LVH (1) and hypertension (1) are all regarded as likely mechanisms leading to LA remodelling in this population. LAV enlargement predicts mortality over and above LVH and LV dysfunction and other risk factors in ESRD (1) and LAV monitoring provides prognostic information beyond that of these major echocardiographic markers of high cardiovascular risk (2). These observations, which are germane to findings in population-based studies (22), mildly hypertensive individuals (23) and in patients with heart disease (24), coherently establish LAV as a relevant prognostic factor in ESRD and expand the information that can be obtained by standard echocardiography. Notwithstanding this technique is formally recommended by practice guidelines (3) as an important tool for risk stratification in chronic kidney disease, because of cost and logistic reasons it is applied less than needed in many dialysis centres. To surrogate information by echocardiography, several studies investigated the diagnostic and prognostic value for major clinical events of biomarkers of left ventricular mass and function (25). The relationship between left atrial volume and ANP was en studied in detail in a recent population-based survey (26). However, until now there is virtually no information on cardiac natriuretic peptides as biomarkers of LAV and as predictors of LAV enlargement over time in the ESRD population.

Cardiac natriuretic peptides and NE as biomarkers of LAV in ESRD patients

Under physiological conditions the atria represent the most rich source of circulating ANP while BNP synthesis in this cardiac chamber is relatively small (27). In the presence of volume overload, LVH and/or LV dysfunction – i.e. in patho-physiological situations which commonly occur in ESRD - the synthesis of BNP in the left atrium increases substantially and

matches that of ANP (28-29). Due to the lack of renal clearance these peptides accumulate in ESRD. However, it was shown that also in ESRD plasma levels of ANP and BNP mainly depend on alterations in LV mass and function rather than on reduced renal clearance (5). As to the relative prognostic value of these peptides, BNP may be a more accurate marker of increased intra-cavitary pressure and LV dysfunction (30) than ANP because the induction of BNP mRNA is faster (31) and more responsive to stretch (32) than that of ANP. Accordingly, we found that BNP displayed a stronger association with LAV changes over time than ANP. In particular, the additional predictive value for progressive LAV enlargement of BNP above established risk factors was higher (+12%) than that provided by ANP (+8%). Notably, this additional prognostic information by BNP was also superior to that of echocardiographic parameters of LV mass (+5%) and function (+8%). Our study did not include direct measurements of a fundamental determinant of LAV, i.e. extracellular volume. Observations based on a very large dialysis population have recently shown that inter-dialysis weight gain, a surrogate of volume expansion, predicts death and cardiovascular events in ESRD (33). We found that this surrogate predicts progression in LAV enlargement thus implicating volume expansion in progressive LAV enlargement and associated alterations in LV mass and function in ESRD.

NE is a marker of sympathetic activity and a risk factor for cardiovascular events in ESRD patients (34). This neurotransmitter promotes myocardial cell hypertrophy in vitro (35) and it was shown that ESRD patients with high NE have a high risk of concentric LVH (7). We found that LAV changes were more pronounced in patients with concentric LVH than in those without suggesting that high NE is a critical element in the chain of events leading to LAV enlargement via concentric LVH and diastolic dysfunction.

An important question addressed in our study was whether a strategy based on single biomarkers is preferable to one based on multiple biomarkers for predicting the evolution of LAV over time. We found that the simultaneous measurements of BNP and NE was marginally superior (P=NS) to the ANP-BNP combination and that both combinations were better than isolated measurements of the same compounds. BNP and ANP are strongly interrelated and therefore provide overlapping predictive information. The small gain in predictive power by the BNP-NE combination may depend on the fact that these biomarkers reflect partially non overlapping pathophysiological pathways leading to LAV enlargement. Whether the additional information provided by the multiple biomarkers strategy translates into better clinical outcomes is an important clinical question that remains to be investigated in future clinical trials.

Our study has limitations. First, although the bootstrap procedure is a powerful technique to internally validate prediction models (16), our findings need to be externally validated in other cohorts of ESRD patients. Second, the use of a single measurement of biomarkers predicting LAV changes may have generated “regression dilution bias” (36). However, this bias generally leads to an underestimation, rather than to an overestimation, of the true relationship between a predictor and a given outcome variable.

Perspectives

Plasma BNP and ANP are strongly related to LAV in the ESRD patients and predict LAV changes over time in these patients. Because an increased LAV underlies diastolic dysfunction and/or volume overload, i.e. potentially modifiable risk factors, the measurement of the plasma concentration of these compounds might be useful for risk stratification and for guiding treatment in dialysis patients.

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Chapter 3.2

Smoking and Hyperparathyroidism in Patients with End Stage Renal Diseases

ABSTRACT

Smoking is associated with hyperparathyroidism in the elderly in the general population and nicotine, the main component of smoking, stimulates PTH release in experimental models. Smoking is a persisting problem in patients with end stage renal disease (ESRD), however the association between smoking and PTH has never been specifically examined in these patients. We investigated the relationship between smoking and hyperparathyroidism in a well characterised group of 161 non-diabetic dialysis patients.

Sixty-four patients (40%) were smokers. Heavy smokers had higher intact PTH (median: 280 pg/mL) and PTH_{1-84} (188 pg/mL) when compared to light smokers (180 pg/mL and 95 pg/mL) and non-smokers (169 pg/mL and 95 pg/mL). In a multiple regression analysis smoking was independently associated to intact PTH (β=0.29, P=0.002) and PTH_{1-84} (β=0.29, P=0.002). Fifty-six patients out of 161 (i.e. 35%) were classified as having hyperparathyroidism. In a multiple logistic regression model the odds of hyperparathyroidism was about 4 times higher in heavy smokers (odds ratio: 3.88, 95% CI: 1.16-12.92, P=0.027) than in non smokers.

In dialysis patients heavy smoking is independently associated to high levels of intact PTH and PTH_{1-84}. Further observational, mechanistic and interventional studies are needed to assess the nature (causal or non causal) of these links in ESRD.

Key words: dialysis, hyperparathyroidism, parathormone, smoking.

INTRODUCTION

On epidemiological scale, cigarette smoking is a major risk factor for human health being causatively implicated in various chronic disease states such as cancer, chronic obstructive pulmonary disease, cardiac ischemia and other cardiovascular (CV) complications (1-3). In patients with end stage renal disease (ESRD) cigarette smoking is mainly regarded as a risk factor for CV complications (4). In theory the detrimental health effect of smoking in ESRD may also extend to one of the most common endocrine alterations in this condition, namely hyperparathyroidism. Indeed, direct and indirect mechanisms exist whereby this environmental risk factor may affect parathyroid gland functioning. In an aggregate analysis of two large hemodialysis populations in the United States (5), smoking resulted to be a direct and independent correlate of hyperphosphatemia, a major trigger of hyperparathyroidism. Furthermore smoking was associated with lower 25-hydroxy-vitamin and higher PTH levels in a study in elderly women (6). As to the possibility of a direct effect of smoking on PTH, it was shown that nicotine increases parathormone (PTH) synthesis and/or release (7-8). In an experimental study in sheep brain (7) nicotine induced a 40% increase in PTH concentration while no such an effect was observed in the control experiment (7). Furthermore, nicotine markedly accumulates in dialysis patients who smoke thus exposing them to a higher risk for the complex effects on health of this alkaloid (9). Notwithstanding the pervasive nature of hyperparathyroidism in ESRD, to date there is no study specifically investigating the relationship between smoking exposure and PTH levels in ESRD.

With this background in mind we undertook a proof of concept, cross sectional study aimed at exploring the relationship between tobacco smoking and PTH as measured by two separate assays targeted at the whole molecule as well as at the intact molecule of this hormone in a well selected group of non diabetic, non parathyroidectomised, ESRD patients who had been never exposed to aluminium and beta-blockers.

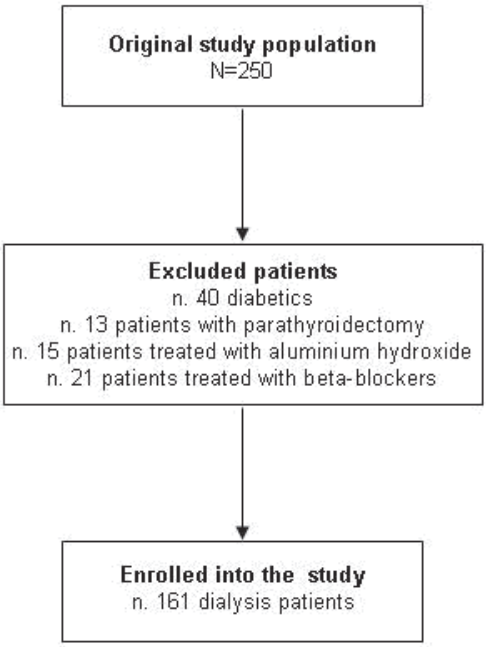
PATIENTS AND METHODS

The protocol was in conformity with the ethical guidelines of our institution and informed consent was obtained from each participant.

Patients

The original study population considered for this study was composed by two-hundred and fifty patients, all Caucasians. By protocol we excluded 40 diabetics, 13 patients with parathyroidectomy, 15 patients who were being or had been treated with aluminium hydroxide and 21 patients on chronic treatment with beta-blockers because all these conditions may independently influence bone turnover and/or PTH secretion (Fig. 1). We specifically excluded

Figure 1. Enrolment scheme of the study.



patients on beta-blockers because these drugs have well characterised suppressive effects on PTH secretion (10). Thus one hundred and sixty-one dialysis patients [131 on hemodialysis (HD) and 30 on chronic ambulatory peritoneal dialysis (CAPD), 93 males and 68 females] were recruited in this study (Table 1). Their mean age was 62.0±15.7 years and the median duration of dialysis treatment was 40.0 months (inter-quartile range 14.0- 88.7 months). Haemodialysis patients were being treated thrice weekly with standard bicarbonate dialysis [Na 138 mMol/L, HCO_3 35 mMol/L, K 1.5 mMol/L, Ca 1.25 mMol/L, Mg 0.75 mMol/L] either by high flux hemodialysis and 1.1-1.7 m^2 hollow-fiber or flat plat dialysers. Dialysis fluid was produced by a reverse osmosis system and Aluminium never exceeded 5 µg/L which is well below the safety limit recommended by the European Best Practice Guidelines (11). Patients on CAPD were all on 4 exchanges/day schedule with standard dialysis bags containing 1.75 mMol/L calcium. Eighty-eight patients were on treatment with erythropoietin. One hundred and thirty-nine patients were consuming calcium salts (either calcium carbonate or calcium acetate). Seventy-six patients were being treated with calcitriol. Sixty-four patients out of 161 (i.e. the 40%) were active smokers while the remaining patients had never smoked or quitted smoking at least three years before the study. As previously reported in a large study in elderly (8) the effect of smoking exposure on biomarkers of bone turnover was analysed by dividing patients into three groups: non-smokers, light smokers ($\leq$ 1 packet/day, i.e. $\leq$ 20 cigarettes/day) and heavy smokers (> 1 packet/day, i.e. >20 cigarettes/day).

Table 1 Clinical and biochemical parameters in patients divided on the basis of cigarettes smoking exposure.

		Packets of cigarettes/day			
	Whole group (n=161)	Non-smokers (n=97)	≤ 1 packet/day (n=43)	>1 packet/day (n=21)	P for trend
Age (years)	62.0±15.7	60.5±17.3	62.7±13.4	67.3±11.1	0.07
Duration of smoking exposure (years)	0 (0-30)	…	30 (18-40)	36 (27-47)	<0.001
Duration of RDT (months)	40 (14-89)	43 (14-88)	47 (22-125)	17 (10-38)	0.08
Males n. (%)	93(58%)	36 (37%)	37 (86%)	20 (95%)	<0.001
BMI (Kg/m^2)	24.6±4.1	24.5±4.4	24.4±3.4	25.9±3.9	0.14
On treatment with Ca carbonate or Ca acetate n. (%)	139 (86%)	84 (87%)	37 (86%)	18 (86%)	0.90
On treatment with calcitriol n. (%)	76 (47%)	40 (41%)	25 (58%)	11 (52%)	0.13
On CAPD treatment n. (%)	30 (19%)	14(14%)	10(23%)	6(29%)	0.08
LVMI ($g/m^{2.7}$)	64.4±19.6	62.3±19.2	66.0±19.9	70.5±20.4	0.07
Serum intact PTH (pg/mL)	197 (71-425)	169(56-370)	180(77-525)	280(235-482)	0.03
Serum PTH_{1-84} (pg/mL)	113 (40-300)	95(32-247)	95(44-328)	188(136-331)	0.02
PTH_{1-84}/C-PTH fragment	1.45(1.10-1.87)	1.47(1.13-1.94)	1.26(1.00-1.62)	1.64(1.37-2.53)	0.07
Serum Calcium (mMol/L)	2.27±0.27	2.28±0.26	2.23±0.26	2.28±0.33	0.99
Serum Phosphate (mMol/L)	1.94±0.44	1.97±0.48	1.87±0.35	1.97±0.39	0.96
CRP (mg/L)	7.1(3.4-16.0)	4.7(3.4-14.8)	8.2(3.4-18.3)	9.0(4.0-19.1)	0.26

Data are reported as mean±SD, median and inter-quartile range or as percent frequency, as appropriate.

Atherosclerosis burden and socio-economic status

The burden of atherosclerosis disease was calculated on individual basis as sum of 5 variables defined in categorical terms (0=absence; 1=presence of previous myocardial infarction, stroke, transient ischemic attach, anginal episodes and peripheral artery disease). Socio-economic status was pragmatically evaluated by 4 members of medical staff by using a score estimating the standard of living of dialysis patients as graded into three classes (from poor to high).

Laboratory measurements

All plasma samples were collected in fasting state between 8 a.m. and 10 a.m. Blood sampling was performed during a midweek non-dialysis day for HD patients and at empty abdomen for CAPD patients. Serum calcium and phosphate were measured using standard methods in the routine clinical laboratory (Vitros 750 Analyzer Johnson & Johnson, Hihj Wycombe, UK). Serum intact PTH as well as the whole PTH molecule (PTH_{1-84}) measurements were made by a specific immunoradiometric assays (Scantibody Laboratory Inc, Santee, CA, USA) and carboxyterminal PTH fragment (C-PTH fragment). The ratio between PTH_{1-84} and carboxy-terminal PTH fragment (C-PTH fragment) was calculated as an index of PTH-dependent bone reabsorption activating and inhibiting forces (12). According to K/DOQI guidelines a value

of intact PTH >300 pg/mL was considered indicative of hyperparathyroidism (13). Serum C-Reactive Protein (CRP) was measured as reported elsewhere (14).

Echocardiography

These studies were performed in a non-dialysis day for hemodialysis patients and at empty abdomen for CAPD patients within 2 hours after blood sampling. All echocardiographic measurements were carried out according to the recommendations of the American Society of Echocardiography by an observer unaware of biochemical results. Left ventricular mass (LVM) was calculated according to the Devereux formula and indexed to height$^{2.7}$ (LVMI) (15).

Statistical Analysis

Data are reported as mean±SD, median and inter-quartile range or as prevalence rate. Differences among groups were assessed by P for trend. Variables showing a positively skewed distribution were log transformed (lg_{10}) before the correlation study.

To control for confounding that could not be eliminated by patients selection we tested the independent relationship between smoking and biochemical markers of bone turnover in a multiple linear regression model including age, sex, socio-economic status, treatment modality (HD/CAPD), duration of dialysis, serum calcium, serum phosphate, use of calcium salts (Ca carbonate or acetate), use of calcitriol, atherosclerosis burden, LVMI, body mass index (BMI) and CRP. In addition, to further analyze the association between smoking and hyperparathyroidism we used multivariate logistic regression analysis. Data are expressed as standardised regression coefficients (β), odds ratio and 95% confidence interval (CI) and P values. All calculations were done using a standard statistical package (SPSS for Windows Version 9.0.1, 11 Mar-1999, Chicago, Illinois, USA).

RESULTS

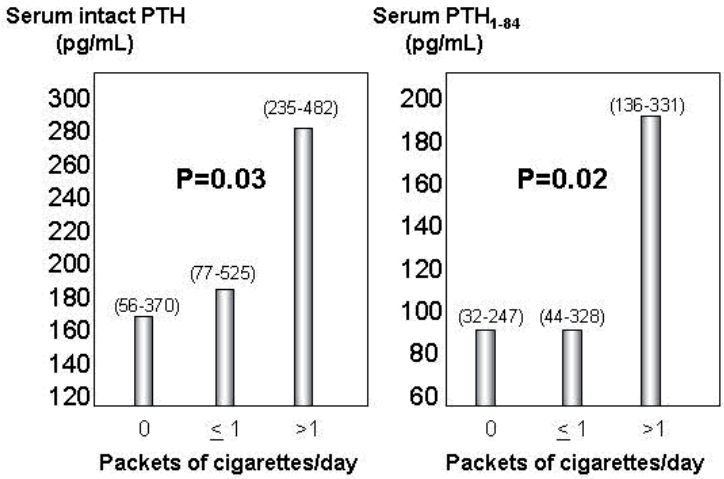
The main demographic, somatometric, clinical and biochemical data of the whole study population and of the patients as grouped on the basis of packets of cigarettes smoked are detailed in Table 1. Sixty-six patients had had background atherosclerotic complications: 38 patients had had one atherosclerotic complication (myocardial infarction in 6 cases, stroke in 1 case, transient ischemic attach in 3 cases, anginal episodes in 23 cases and peripheral artery disease in 5 cases) and the remaining 28 patients had had $\geq$ 2 atherosclerotic complications. As shown in Table 1 patients who smoked > 1 packet/day were more frequently males, had smoked for a longer period and tended to be older, on dialysis for a shorter time and displayed higher LVMI when compared to the remaining patients. The proportions of patients on CAPD and on treatment with calcitriol were higher in heavy/moderate smokers as compared to non smokers but these differences did not attain the statistical significance

(Table 1). The proportion of patuents As shown in Fig. 2 both serum intact PTH and PTH_{1-84} were significantly higher in heavy smokers than in light smokers and non smokers. The three study groups showed similar serum concentrations of calcium and phosphate as well as similar levels of serum CRP. Of note, serum intact PTH and PTH_{1-84} were largely unrelated to atherosclerosis burden (P=0.51 and P=0.64, respectively) and socio-economic status (P=0.79 and P=0.80, respectively).

Association between smoking, serum intact PTH and PTH_{1-84}: multivariate linear regression analysis

Because the association between serum intact PTH and PTH_{1-84} with smoking could be confounded by other risk factors for bone disease, we performed multiple regression analyses adjusting for all potential confounders (Table 2). In these analyses, smoking resulted to be an independent predictor, and a highly significant one, of both serum intact PTH (β=0.29, P=0.002) (Table 2a) and PTH_{1-84} (β=0.29, P=0.002) (Table 2b) being the second factor in rank, after treatment modality, to explain the variability in serum levels of these biomarkers. Further analysis of these associations showed that these links were much stronger in patients with a BMI <25 kg/m^2 (n=93, smoking-intact PTH relationship: β=0.30, P=0.009; smoking-PTH_{1-84}: β=0.30, P=0.008) than in those with a BMI ≥ 25 kg/m^2 (n=68, β=0.19 and β=0.17, respectively). These results did not materially change also in a separate analysis of HD and CAPD patients (data not shown).

Figure 2. Association between smoking exposure with serum intact PTH and PTH_{1-84}.



Data are expressed as median and inter-quartile range.

Table 2 Multiple regression analyses of indicators of serum intact PTH and PTH_{1-84}

a) Dependent variable: serum intact PTH

	β	P
Treatment modality (0=HD; 1=CAPD)	-0.36	<0.001
Smoking	0.29	0.002
Male sex	-0.18	0.04
LVMI	0.18	0.05
Use of calcium carbonate/calcium acetate	-0.13	0.11
Serum calcium	-0.08	0.32
Socio-economic status	0.07	0.41
BMI	0.06	0.46
Atherosclerosis burden	-0.06	0.48
Age	-0.06	0.55
CRP	0.03	0.70
Duration of dialysis	0.02	0.80
Use of calcitriol	-0.02	0.81
Serum phosphate	-0.004	0.96

b) Dependent variable: serum PTH_{1-84}

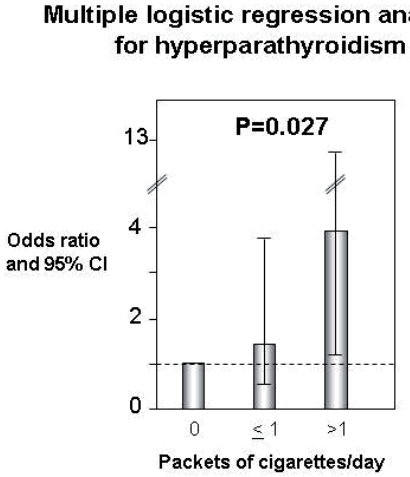
	β	P
Treatment modality (0=HD; 1=CAPD)	-0.36	<0.001
Smoking	0.29	0.002
Male sex	-0.19	0.04
LVMI	0.17	0.06
Use of calcium carbonate/calcium acetate	-0.12	0.14
Serum calcium	-0.10	0.22
BMI	0.06	0.47
Atherosclerosis burden	-0.06	0.47
Socio-economic status	0.05	0.50
CRP	0.03	0.67
Age	-0.04	0.70
Serum phosphate	-0.01	0.89
Use of calcitriol	-0.007	0.93
Duration of dialysis	0.006	0.94

Data are expressed as standardised regression coefficients (β) and P values.

Association between smoking and hyperparathyroidism: multivariate logistic regression analysis

In the aggregate, 56 patients out of 161 (i.e. 35%) had a serum intact PTH >300 pg/mL and were classified as having hyperparathyroidism. In a multiple logistic regression model, including the same set of variables listed in Table 2, the odds of hyperparathyroidism was highest in heavy smokers (odds ratio: 3.88, 95% CI: 1.16-12.92, P=0.027) and lower in mild smokers and in non smokers (reference category) (Fig. 3).

Figure 3. Odds ratio for hyperparathyroidism associated to packets of cigarettes/day (data were adjusted for variables listed in Table 2).



Data are odds ratio and 95% CI.

DISCUSSION

This study associates heavy cigarette smoking with high circulating levels of serum intact PTH and PTH_{1-84} in a carefully selected dialysis population of non-diabetic patients, never exposed to aluminium and beta blockers.

Hyperparathyroidism in ESRD

Hyperparathyroidism is a frequent complication is ESRD patients (16) and represents an important treatment target (17-18). Hyperparathyroidism and high bone turnover in ESRD are currently attributed to a complex series of risk factors including hyperphosphatemia, hypocalcemia, 1,25 dihydroxyvitamin D3 deficiency, decreased expression of calcium and vitamin D receptors and parathyroid hormone resistance (19). Such a complication has a negative impact not only on bone structure but also on a variety of extra-osseous complications such as dilated cardiomyopathy (20), vascular damage (21,22) and fistula access thrombosis (23).

Smoking and hyperparathyroidism

While factors related with alterations in calcium and phosphate metabolism have been intensively investigated only scanty attention has been focused on environmental factors potentially affecting parathyroid gland function in ESRD patients. In particular, the association between cigarette smoking and biomarkers of hyperparathyroidism has never been specifically tested in these patients. In our study two well recognised markers of bone turnover like serum intact PTH and PTH_{1-84} resulted to be significantly higher in heavy smokers (i.e. patients who smoked > 1 packet of cigarettes/day) than in the remaining patients (i.e. light smokers

or non-smokers) and these associations became stronger in multivariate linear regression analyses taking into account a series of potential confounders (Table 2).

In non uremic populations the PTH concentration has been reported to be lower (24-27), similar (28) or higher (6) in smokers than in non-smokers. In keeping with the observation by Rapuri et al. in elderly women in the general population (6) we found that the association between smoking and high serum PTH was stronger in heavy smokers suggesting that this link is non-linear being particularly evident at high levels of exposure (> 1 packet of cigarettes/day). In a study in two large hemodialysis populations in the United States in which smoking resulted to be a direct and independent predictor of hyperphosphatemia (5), a well recognised trigger of hyperparathyroidism in ESRD patients. Smokers may be less compliant to dietary restrictions and phosphate binders use and it well documented that smokers on dialysis frequently skip dialysis and show higher inter-dialysis weight gain as compared to non-smokers (29). However, no association between phosphate and PTH emerged in our study. Effect modification by other risk factors is an important issue to be considered to explain apparent discrepancies among studies in different populations. For example, in a study in 719 men (24) PTH was unrelated to smoking exposure but an analysis stratified by body weight revealed that among men with body weight <75 Kg serum intact PTH was by the 16% higher in smokers than in non-smokers, a difference of high statistical significance (P=0.005). Similarly, in our study the association between smoking and serum intact PTH and PTH_{1-84} in dialysis patients was prominent in patients with a BMI <25 kg/m^2 but much attenuated in those with BMI >25 kg/m^2. Reduced body mass may interact with smoking in aggravating the effect of hyperparathyroidism and high bone turnover because in post menopausal women (30) the negative impact of smoking on bone loss is evident only in women in the first tertile of body fat. Malnutrition is a common complication in patients with ESRD (31) which may help to explain the fact that the association between heavy smoking and high PTH was stronger in patients with BMI <25 kg/m^2.

In theory the effect of smoking on PTH may be the expression of a direct action of nicotine on parathyroid glands (7,8), a possibility also suggested by the fact that nicotine accumulates in dialysis patients (9). Pre and post smoking serum nicotine levels are 4 to 5 times higher in smokers dialysis patients than in well matched control smokers and plasma nicotine post smoking attains levels as high as 133 ng/ml (9). High-dose nicotine administration in rats increases both serum phosphate and PTH (8) and in an experimental study in sheep brain, nicotine induced a clear-cut increase (+40%) in PTH concentration (7). Since nicotine has a stimulating effect on cyclic adenosine monophosphate (cAMP) secretion (32) and because an analogue of cAMP (dibutyryl cAMP) stimulates PTH synthesis (33) it can be speculated that the nicotine-induced PTH increase might be mediated via the nicotine-cAMP pathway. Although the hypothesis that the link between hyperparathyroidism and smoking underlies a casual mechanism appears plausible, the possibility that this association may be the mere effect of confounding factors (like malnutrition, vitamin D deficiency and

compliance to therapies) cannot be easily dismissed. Because hyperparathyroidism is a risk factor for cardiovascular disease it is possible that the association found in the present study reflects the high CV burden of ESRD and the fact that smoking is a recognised risk factor for CV complications in ESRD patients (4). However, we found that the odds for high PTH associated with heavy smoking was unmodified in models adjusting for atherosclerosis burden and left ventricular hypertrophy. As for the lower levels of PTH in CAPD as compared to HD patients (Table 2), it can be speculated that treatment modality-related factors (like Calcium concentration in the dialysate) can affect hyperparathyroidism in the dialysis population.

Our study has obvious limitations. First, it is based on a cross-sectional design. Second, concomitant therapies aimed at controlling hyperparathyroidism and associated calcium phosphate disturbances might have influenced our results in an unpredictable manner and may in part explain the lack of significant relationships between PTH and circulating levels of calcium and phosphate. Although we adjusted for calcium salts and calcitriol treatment, such an adjustment may not adequately account for the effect of these drugs on serum levels of PTH. Third, we did not measure circulating levels of nicotine, the main factor we suspected to increase PTH in ESRD patients. A strength of our study was that, by patients selection and multivariate modelling, we controlled for all major factors responsible for high bone turnover and hyperparathyroidism in ESRD and that we adjusted both for atherosclerosis burden, the socio-economic status and left ventricular hypertrophy which are three main recognised confounders for the interpretation of the effect of smoking.

Overall, our study is “hypothesis-generating” rather than “hypothesis-testing” and observational (follow-up and longitudinal), mechanistic and interventional studies are needed to confirm the link between smoking and high PTH and to assess the nature (causal or non causal) of this link in ESRD patients.

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Chapter 3.3

Aging and Left Ventricular Mass and Function in patients with End Stage Renal Diseases

ABSTRACT

Left ventricular hypertrophy (LVH) and LV systolic dysfunction are frequent complications of end stage renal disease (ESRD) and represent strongest risk factors for death and adverse cardiovascular (CV) outcomes in ESRD patients. While the risk of cardiomyopathy in the dialysis population is well defined, the relationship between senescence with LV mass (LVMI) and LV systolic dysfunction (as assessed by mid-wall fractional shortening, mwFS) has never been analysed into detail in ESRD. In this study we sought to identify age-related factors responsible for cardiomyopathy in 254 patients on chronic dialysis.

At echocardiography, 196 patients displayed LVH (77%) and 123 (48%) had LV systolic dysfunction. On univariate analysis, age was related directly with LVMI ($r=0.33$, $P<0.001$) and inversely with mwFS ($r= - 0.23$, $P<0.001$) and 10 years increase in age was associated with 4.2 $g/m^{2.7}$ increase in LVMI and -0.5% decrease in mwFS. Age-related risk factors for both LVMI and mwFS were albumin, pulse pressure, CV comorbidities and C-Reactive Protein. Haemoglobin resulted to be an age-dependent risk factor for LVMI while heart rate and diabetes were age-dependent risk factors for mwFS. After adjusting for age-related risk factors, the predictive value of age for cardiomyopathy drastically reduced (-67% for LVMI and -61% for mwFS) and 10 years increase in age was associated to a very modest and not significant change in LVMI (+1.4 $g/m^{2.7}$) and mwFS (-0.2%).

In ESRD patients the relationship between age and cardiomyopathy is largely dependent on age-related risk factors. Interventions focused on modifiable risk factors linked to age (like malnutrition/inflammation and arterial rigidity) could attenuate the detrimental effect of aging on cardiovascular risk in the dialysis population.

Key words: aging, dialysis, left ventricular hypertrophy, left ventricular systolic function.

INTRODUCTION

In the last decades, improvements in dialysis techniques and advances in treatment of hypertension, anaemia, cardiovascular (CV) diseases and dialysis-related complications, translated into an important increase of life expectancy in patients with end stage renal diseases (ESRD) (1-2). On the other hand, in European countries the proportion of incident patients with ESRD aged over 65 years increased from 22% in 1980 to 60% in 2005 (3). The main consequence of these demographic changes has been an important increase of the mean age of the dialysis population (2). In both health and in disease, aging *per se* and/or by increasing the time of exposure to other risk factors triggers important effects on CV system. It was remarked that the risk of CV death of a young man (<30 years) on dialysis equals that of a healthy man over 85 years in the general population suggesting that the pathogenetic processes linked to aging may be accelerated in dialysis patients.

Left ventricular hypertrophy (LVH) and LV systolic dysfunction are almost universal complications of end stage renal disease (ESRD) and represent strongest risk factors for death and adverse CV outcomes in these patients (4-5). While the risk of cardiomyopathy in the dialysis population is well defined (4-5), the relationship between age with LV mass and function has never been analysed into detail in ESRD patients. The issue is of importance because cardiomyopathy in ESRD may be in part due to a specific cluster of risk factors linked to senescence.

With this background in mind we designed a study aimed at investigating by statistical modelling the main age-related factors responsible for cardiomyopathy in ESRD patients.

METHODS

Protocol

The protocol was in conformity to the ethical guidelines of our Institutions and informed consent was obtained from each participant. All blood samples for laboratory tests were taken during a mid-week non-dialysis day for haemodialysis patients and at empty abdomen for CAPD patients, between 8 A.M. and 1 P.M.

Patients and controls

We studied an incident-prevalent cohort of 254 dialysis patients (144 M and 110 F) who had been on regular dialysis treatment (RDT) for at least 6 months (median duration of RDT; 42 months; inter-quartile range: 18-97 months). The enrolment criteria in this cohort were no history of congestive heart failure [defined as dyspnea in addition to two of the following conditions - raised jugular pressure, bibasilar crackles, pulmonary venous hypertension or interstitial oedema on chest X ray, requiring hospitalization or extra ultra-ultrafiltration], left ventricular ejection fraction >35% and no inter-current or terminal illnesses. Haemodialysis

patients (n=203) were treated thrice weekly with standard bicarbonate dialysis (Na 138 mMol/L, HCO3 35 mMol/L, K 1.5 mMol/L, Ca 1.25 mMol/L, Mg 0.75 mMol/L) either with cuprophan or semi-synthetic membranes (dialysis filters surface area: 1.1-1.7 m^2). The average urea Kt/V in these patients was 1.22±0.27. The remaining 51 patients were on CAPD (weekly Kt/V: 1.67±0.32).

Laboratory measurements

Blood sampling was performed after an overnight fast between 8.00 a.m. and 10.00 a.m. always during a mid-week non-dialysis day for haemodialysis patients and at empty abdomen for CAPD patients. After 20-30 min of quiet resting in semi-recumbent position samples were taken into chilled EDTA vacutainers, placed immediately on ice, centrifuged within 30 min at -4°C and the plasma stored at -80°C before assay. Serum lipids, albumin, parathormone (PTH), calcium, phosphate, and haemoglobin measurements were made using standard methods in the routine clinical laboratory. The plasma concentrations of CRP and homocysteine were measured according to standard methods described elsewhere (6-7).

Echocardiography

These studies were performed in a non-dialysis day for haemodialysis patients and at empty abdomen for CAPD patients within 2 hours after blood sampling. In healthy subjects echocardiographic measurements were performed in the morning hours. Left ventricular mass (LVM) was calculated according to the Devereux formula and indexed to height$^{2.7}$ (LVMI) (8). LVH was defined by a LVMI >47 g/$m^{2.7}$ in women or >50 g/$m^{2.7}$ in men. The height-based indexing of LVM minimises any potential distortion attributable to extra-cellular volume expansion (body surface area indexing being weight-sensitive) and provides more accurate prognostic information than that by body surface area in the dialysis population (4). Systolic function was measured by midwall fractional shortening (mwFS) according to the method of Shimizu et al. (9) as described in full detail by de Simone et al. (10). We elected to use this parameter because its high reliability and because in patients with ESRD it is an indicator of LV systolic dysfunction more sensitive than standard ejection fraction (7). A value of mwFS <14% was considered indicative of LV systolic dysfunction (5).

Statistical analysis

Data are expressed as mean ± standard deviation, median and inter-quartile range (IQR) or as percent frequency, as appropriate. Comparisons between two groups were made by T-Test, Mann-Whitney Test or Chi Square test and among three or more groups by P for trend. The relationship between continuous variables was investigated by Pearson product moment correlation coefficient (r) and P value. Variables having a positively skewed distribution were log transformed (lg_{10}) before the correlation study.

The independent relationship between aging and LVMI and mwFS was investigated by univariate and multivariate linear regression analyses. Multivariate models adjusted for age-related factors responsible for cardiomyopathy in ESRD patients, that is variables a) which were related (P<0.10) with both, exposure (age) and study outcomes LVMI and mwFS) b) which were not an effect of exposure and c) which were not in the causal pathway between the exposure (age) and study outcomes (11). Tested covariates included Framingham risk factors (age, sex, smoking, diabetes, cholesterol and blood pressure), background CV complications, anti-hypertensive treatment, factors peculiar to ESRD (dialysis vintage, albumin, haemoglobin and calcium*phosphate), CRP and homocysteine. The increase (or decrease) in age-related risk factors of LVH and LV systolic dysfunction having the same effect on cardiomyopathy as a 10-years increase in age was calculated by using the regression coefficients derived from multiple regression models. To infer the involvement of traditional and non traditional risk factors in the patho-physiological pathway linking senescence and LV mass and function in ESRD patients we adopted the approach by Kraemer et al. (12). Data are expressed as regression coefficient (slope), standardised regression coefficient (β) and P values. All calculations were done by a standard statistical package (SPSS for Windows Version 9.0.1, 11 Mar-1999, Chicago, Illinois, USA).

RESULTS

The main clinical and biochemical data of ESRD patients as divided according to age tertiles are detailed in Table 1. To identify age-related risk factors in ESRD we performed categorical and continuous data analyses. Patients in the third age tertile had higher pulse pressure, heart rate and CRP and lower diastolic arterial pressure, phosphate, haemoglobin and albumin and have been on dialysis for a shorter time when compared to those in the second and in the first age tertiles. CV comorbidities increased in close parallelism with aging, so that the proportion of patients with background CV complications was about two times higher in the third (65.1%) than in the first (29.1%) age tertile. Patients in the first age tertile were those with the lowest proportion of diabetics (4.7%). No difference in other clinical and biochemical data were observed among age groups. These categorical analyses went along with correlation analyses considering age as continuous variable (see Table 1, last column).

Aging, LV hypertrophy and systolic dysfunction: univariate analysis

At echocardiography, 196 patients displayed LVH (77%) and 123 (48%) had LV systolic dysfunction. Patients in the third age tertile had higher LVMI and lower mwFS as compared to those in the remaining two age tertiles (Table 1) and a correlation analysis with age as continuous variable confirmed these associations (Fig. 1). On crude analyses, 10 years increase in age was associated to 4.2 $g/m^{2.7}$ increase in LVMI and to -0.5% decrease in mwFS.

Table 1 Main clinical and biochemical characteristics of patients divided accordingly to age categories.

	Age categories (tertiles)				
	<56 years	56-68 years	>68 years	P for trend	r(P)
Dialysis vintage (months)	47(20-131)	43(18-101)	30(14-60)	0.02	-0.15(0.02)
Male sex n. (%)	56(65.1%)	42(49.4%)	46(55.4%)	0.20	-0.06(0.37)
Smokers n. (%)	36(41.9%)	34(40.0%)	37(44.6%)	0.73	0.12(0.06)
Diabetics n. (%)	4(4.7%)	19(22.4%)	14(16.9%)	0.02	0.14(0.02)
With CV co-morbidities n. (%)	25(29.1%)	43(50.6%)	54(65.1%)	<0.001	0.36(<0.001)
On anti-hypertensive treatment n. (%)	44(51.2%)	34(40.0%)	33(39.8%)	0.13	-0.05(0.41)
On treatment with EPO n. (%)	48(55.8%)	44(51.8%)	43(51.8%)	0.60	-0.06(0.34)
Systolic pressure (mmHg)	133±22	133±24	134±22	0.70	0.02(0.70)
Diastolic pressure (mmHg)	79±13	74±12	72±11	<0.001	-0.24(<0.001)
Pulse pressure (mmHg)	54±15	59±16	62±18	0.002	0.21(0.001)
Heart rate (beats/min)	84±12	81±10	77±13	<0.001	-0.21(0.001)
Total cholesterol (mg/dL)	200±58	211±54	209±51	0.33	0.08(0.22)
Triglycerides (mg/dL)	163±81	188±98	172±82	0.49	0.05(0.44)
HDL cholesterol (mg/dL)	42±13	41±10	41±13	0.85	0.03(0.70)
LDL cholesterol (mg/dL)	126±49	132±47	133±45	0.36	0.07(0.31)
Calcium (mg/dL)	4.6±0.5	4.5±0.5	4.5±0.6	0.21	-0.10(0.11)
Phosphate (mg/dL)	6.1±1.4	5.9±1.4	5.6±1.3	0.01	-0.19(0.003)
PTH (pg/mL)	164(65-353)	132(62-407)	142(48-271)	0.45	-0.06(0.42)
Haemoglobin (g/L)	111±19	106±17	101±19	0.001	-0.16(0.009)
Albumin (g/L)	42±6	40±5	38±5	<0.001	-0.35(<0.001)
CRP (mg/L)	4.3(3.4-11.8)	8.3(3.4-18.6)	8.3(3.4-22.7)	0.01	0.22(0.001)
Homocysteine (μMol/L)	30.8(20.7-47.9)	26.2(19.4-39.8)	25.1(18.7-35.9)	0.09	-0.14(0.03)
LVMI ($g/m^{2.7}$)	57±18	65±19	70±20	<0.001	0.33(<0.001)
mwFS (%)	14.6±3.6	14.3±3.2	13.5±2.9	0.009	-0.23(<0.001)

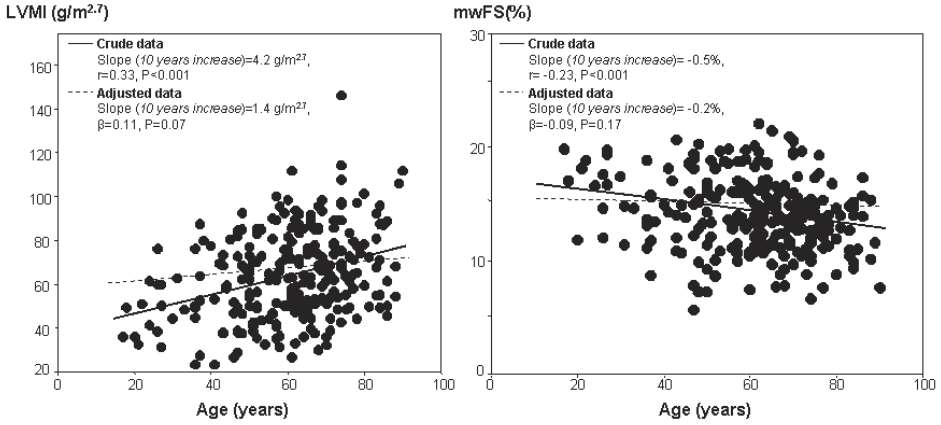
Data are expressed as mean± SD, median and inter-quartile range or as percent frequency, as appropriate. In the last column the correlation coefficient of age as continuous variable with all risk factors listed in **Table 1** is also reported.

Age-related risk factors for LVMI and mwFS were albumin, pulse pressure, CV comorbidities and CRP. Haemoglobin resulted to be an age-dependent risk factor for LVMI while heart rate and diabetes age-dependent risk factors for mwFS.

Aging, LV hypertrophy and dysfunction: multiple linear regression analysis

To identify the relevance of age-dependent risk factors for deterioration in LV mass and function in ESRD we adjusted the relationship between senescence and cardiomyopathy for age-associated risk factors of LVH and LV systolic dysfunction. After adjusting for age-related risk factors, the predictive value of age for cardiomyopathy drastically reduced (-67% for LVMI and -61% for mwFS, see Table 2) and 10 years increase in age was associated only to

Figure 1. Crude (continuous line) and adjusted (dotted line) relationships between age and LVMI and mwFS in ESRD patients.



Data are expressed as regression coefficient (slope correspondent to 10 years increase in age), correlation coefficient and P value.

Table 2 Multiple linear regression analyses of the relationship between aging and LVMI (**a**) and mwFS (**b**).

a) Dependent variable: LVMI

	Age-LVMI link		
	Crude analysis	*Adjusted analysis*	
	r(P)	**β**	**P**
Age	**0.33(<0.001)**	**0.11**	**0.07**
Albumin		-0.31	<0.001
Pulse pressure		0.26	<0.001
CV comorbidities		0.12	0.04
Haemoglobin		-0.10	0.07
CRP		-0.04	0.43

b) Dependent variable: mwFS

	Age-mwFS link		
	Crude analysis	*Adjusted analysis*	
	r(P)	**β**	**P**
Age	**-0.23(<0.001)**	**-0.09**	**0.17**
Albumin		0.26	<0.001
Heart rate		-0.25	<0.001
CV comorbidities		-0.14	0.02
Pulse pressure		-0.13	0.04
Diabetes		-0.11	0.07
CRP		-0.03	0.55

Data are expressed as Pearson product moment correlation coefficient (r), standardised regression coefficient (β) and P value.

a very modest and not significant change in LVMI (+1.4 g/m$^{2.7}$) and mwFS (-0.2%) (Fig. 1). In multiple linear regression models (Table 2), the predictive value of aging for LVMI and mwFS was mostly captured by albumin, pulse pressure and CV comorbidities (Table 2). Heart rate

only predicted mwFS. To put these in perspective, decreases of 13.3 g/L and 1.2 g/L in serum albumin and increases of 5 mmHg and 8 mmHg in pulse pressure had the same association with LVMI and mwFS, respectively, as a 10-years increase in age.

DISCUSSION

This study shows that in ESRD patients the relationship between age and cardiomyopathy is largely dependent on age-related risk factors and suggests that interventions focused on modifiable risk factors linked to age (like malnutrition/inflammation and arterial rigidity) could attenuate the detrimental effect of aging on cardiovascular risk in the dialysis population.

Aging and cardiovascular risk in ESRD patients

Aging is a complex biological process and clinical and epidemiological studies coherently indicate that the cardiovascular system is an important target of senescence in both health and disease (13-16). Left ventricular (LV) hypertrophy and LV systolic dysfunction are pervasive alterations in ESRD patients and represent important risk factors for death and cardiovascular events in the dialysis population (4-5). In the last decades, a variety of risk factors for LVH and LV dysfunction have been identified in dialysis patients (17) but no study specifically focused on age-related risk factors for these alterations in ESRD patients by directly comparing the effect of aging in dialysis patients and in healthy subjects. The issue is of importance because the biological processes linked to aging may be to be accelerated in ESRD patients (18) and because the pathophysiological pathways underlying age-related alterations in LV mass and function in ESRD patients are not completely clarified yet. In this study, on crude analysis age resulted to be a significant correlate of LV hypertrophy and LV dysfunction in ESRD patients. Albumin, pulse pressure, haemoglobin, heart rate, diabetes and CV comorbidities were associated with both age and LV mass and/or LV systolic function. These parallel associations suggest that these well recognised risk factors for cardiomyopathy in ESRD may be the main drivers of the age-dependent increase in LV mass and dysfunction in this population. To test this hypothesis we entered these factors in the multiple regression models aimed at investigating the variability in LV mass and function. Remarkably, after the inclusion of these factors into these models, age was no longer a significant predictor of LVMI and mwFS suggesting that the link between aging and alterations in LV mass and function was largely dependent on these age-related risk factors. Among these factors, albumin (a marker of malnutrition/inflammation) and pulse pressure (a marker of arterial stiffness) emerged as the strongest correlates of LVMI and mwFS. These observations are in keeping with the results of previous studies indicating serum albumin (19-21) and pulse pressure (22) as strong and independent predictors of cardiovascular morbidity and mortality in the dialysis population. Thus, the high

prevalence of cardiac alterations related to aging in dialysis patients largely depend on a specific cluster of risk factors including malnutrition/inflammation and arterial rigidity.

Study limitations

This study has limitations. The cross-sectional design precludes the possibility to draw definitive conclusions about the nature (causal/not causal) of the relationships we found. The second limitation is that we did not compare the link between senescence and cardiomyopathy in ESRD patients and in a large, representative sample, of the general population. Strengths of our study are: 1) it is the only one specifically investigating the relationship between aging and cardiomyopathy in ESRD; 2) the robustness of statistical methods applied to test the study hypotheses

Conclusions

In ESRD patients the relationship between age and cardiomyopathy is largely dependent on age-related risk factors. Interventional studies focused on modifiable risk factors linked to age (like malnutrition/inflammation and arterial rigidity) could attenuate the effect of aging on cardiovascular system in the dialysis population.

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Chapter 4

Risk factors of mortality and cardiovascular events in patients with end stage renal diseases (ESRD)

Chapter 4.1

Left Atrial Volume in End Stage Renal Disease: A Prospective Cohort Study

ABSTRACT

End stage renal disease (ESRD) is a high risk condition and left ventricular hypertrophy (LVH) is the strongest risk factor in this population.

Since the prognostic value of left atrial (LA) size in ESRD is still unknown, we performed a prospective cohort study aimed at testing the prognostic value of LA volume in a cohort of 249 ESRD patients.

Both un-indexed and indexed LA volume (LAV) was significantly higher in dialysis patients than in healthy subjects (P<0.001). On multivariate analysis only left ventricular mass index (LVMI), LV ejection fraction (LVEF), E/A ratio and anti-hypertensive treatment maintained an independent association with LAV. During the follow-up 113 patients died. LAV added significant prognostic power to a multivariate Cox model of all-cause death and the model based on height$^{2.7}$ provided the best data fit. Notably, this index maintained an independent predictive value for death (P=0.03) also when LVMI and LVEF were jointly forced into the Cox's model. Neither crude nor BSA-adjusted LAV had an independent association with death when tested into the Cox model including LVMI and LVEF.

In patients with ESRD, LAV indexed for height$^{2.7}$ displays prognostic value beyond and above that provided by LV mass and function.

Key words: atrial volume, cardiovascular risk, dialysis, epidemiology, LVH, survival analysis.

INTRODUCTION

Echocardiography provides fundamental information for risk stratification in patients with end stage renal disease (ESRD). Indeed left ventricular mass index (LVMI) as measured by this technique is the strongest predictor of death and adverse cardiovascular (CV) outcomes in the dialysis population[1;2]. Both the study of left ventricular (LV) geometry[3] and LV systolic function[4] give additional information for risk stratification in these patients. On the other hand, echocardiography allows detailed studies of atrial chambers. The left atrium (LA) in particular can be reliably measured and its determinants are indeed well defined in population based studies[5] and in patients with heart diseases[6]. The estimation of LA size is considered as an adjunctive, relevant parameter which may enrich the prognostic usefulness of echocardiographic studies[7;8] and the structural and functional correlates of this measurement have been recently characterized in hypertensive patients[9;10]. Although LA diameter or volume measurements have occasionally been used to monitor changes in circulating volume in ESRD, to our knowledge there is still no large scale, systematic study in ESRD patients aimed at defining the functional correlates of LA volume (LAV) nor its prognostic value in this particular population. The problem is relevant because many factors like extra-cellular volume expansion, valvular heart disease, alterations in ventricular mass and compliance, which are common in dialysis patients, may all contribute to increase LAV. A recent study performed in a small group of dialysis patients has shown that LAV may represent a better marker of diastolic function than mitral inflow Doppler-derived indexes[11].

Notwithstanding recommendations that LA be indexed by body surface area (BSA)[5], in general such measurement is crudely reported in clinical practice and in clinical studies as well. In this regard it is important noting that we have shown that indexing LV mass by height$^{2.7}$ rather than by BSA in ESRD patients is important to maximize the prediction power of this measurement[2]. Whether this applies also to LA dimension is unknown.

With this background in mind we set out a prospective cohort study aimed at identifying the functional correlates of LAV in ESRD patients and at testing the prognostic value of this measurement both in an unadjusted form and adjusted by relevant indicators of body size, i.e. BSA and height.

METHODS

Protocol

The study complied with the Declaration of Helsinki. The protocol was in conformity to the ethical guidelines of our institutions and informed consent was obtained from each participant.

Study Cohort

Two hundred and forty nine patients with ESRD (142 M and 107 F) who had been on regular dialysis treatment (RDT) [199 on haemodialysis (HD) and 50 on chronic ambulatory peritoneal dialysis (CAPD)] for at least 6 months, with left ventricular ejection fraction (LVEF) >35% and without history or clinical evidence of circulatory congestion[12], were eligible for the study. No patient had inter-current acute coronary syndromes and all were in sinus rhythm at the time of the study. One hundred and eighteen patients had had at least one CV event. In particular, 63 patients had had one CV event (myocardial infarction in 8 cases, ECG documented anginal episodes in 29 cases, peripheral artery diseases in 11 cases, arrhythmia in 10 cases, transient ischemic attacks in 4 cases and stroke in 1 case) and the remaining 55 patients had had two or three (n=46) or more than three (n=9) CV complications. No patient had severe valvular heart disease. However 34 patients had mild to moderate valvular heart disease: 4 patients had combined valvular stenosis and insufficiency (mitral in 1 case and aortic in 3 cases); 10 patients had isolated valvular stenosis (mitral in 1 case and aortic in 9 cases) and 20 patients had isolated valvular insufficiency (mitral in 13 cases, aortic in 6 cases and a simultaneous involvement of two valves in 1 case). The main demographic, somatometric, clinical and biochemical characteristics of patients included in the study are detailed in Table 1. Patients represented about the 70% of the dialysis population of two urban areas. The remaining 30% of patients were excluded because of the presence of circulatory congestion or major infections (20%) or because they were hospitalised for inter-current illnesses or for logistic reasons/unwillingness to participate in the study (10%). The prevalence of diabetes mellitus in this cohort was 14% (i.e. 35 patients out of 249).

Hemodialysis patients were being treated thrice weekly with standard bicarbonate dialysis (Na 138 mmol/L, HCO_3 35 mmol/L, K 1.5 mmol/L, Ca 1.25 mmol/L, Mg 0.75 mmol/L) by cuprophan or semi-synthetic membranes (dialysis filters surface area: 1.1-1.7 m^2). Dry weight was targeted in each case to achieve a normotensive edema-free state. The average urea Kt/V in these patients was 1.22±0.27. Patients on CAPD were all on 4 exchanges/day schedule with standard dialysis bags. The average weekly Kt/V in these patients was 1.66±0.32. One-hundred and seven patients were habitual smokers (21±17 cigarettes/day). One hundred and thirty patients were on treatment with erythropoietin. One hundred and ten patients were on anti-hypertensive treatment (77 on mono-therapy with ACE inhibitors, AT-1 antagonists, calcium channel blockers, alpha and beta-blockers, and 33 on double or triple therapy with various combinations of these drugs).

Control Group

As a control group we enrolled a series of 100 healthy subjects matched for age and sex (54±12 years; 52 M and 48 F) who underwent echocardiography at the laboratory of our institution as a part of a medical check-up.

Table 1 Main demographic, somatometric, clinical and biochemical data in the study cohort.

	Survivors (n=136)	Non survivors (n=113)	P
Age (years)	53.7±15.5	68.1±11.2	<0.001
Duration of RDT (months)	38 (15-100)	45(18-94)	0.54
Male sex n. (%)	70 (51%)	72(64%)	0.05
Smokers n. (%)	46 (34%)	61(54%)	0.001
Diabetics n. (%)	10 (7%)	25(22%)	0.001
On anti-hypertensive therapy n. (%)	62 (46%)	48(42%)	0.62
With previous CV events n. (%)	42 (31%)	76(67%)	<0.001
Systolic pressure (mmHg)	132.3±20.8	134.4±24.2	0.48
Diastolic pressure (mmHg)	76.6±11.7	73.2±12.9	0.03
Pulse pressure (mmHg)	55.7±15.2	61.1±17.4	0.01
Heart rate (beats/min)	81.2±10.6	79.9±13.9	0.46
Cholesterol (mMol/L)	5.33±1.40	5.30±1.40	0.89
Haemoglobin (g/dL)	107.2±17.9	104.9±20.4	0.36
Albumin (g/L)	41.5±5.3	38.5±5.7	<0.001
Calcium*Phosphate ($mMol^2/L^2$)	4.54±1.18	4.35±1.16	0.20
CRP (mg/L)	4.7 (3.4-11.8)	11.7(4.3-22.8)	<0.001
Homocysteine (µMol/L)	26.8 (19.2-41.6)	27.0(21.1-40.9)	0.91
LVMI ($g/m^{2.7}$)	57.5±16.9	72.0±20.3	<0.001
LV Ejection Fraction (%)	61.0±54.6	54.6±10.1	<0.001
Left atrial volume (ml)	35.8±15.5	43.8±21.0	0.001
Left atrial volume (ml/m^2)	21.5±9.3	26.3±12.5	0.001
Left atrial volume ($ml/m^{2.7}$)	9.6±4.2	12.6±6.3	<0.001
Left atrial diameter (mm)	36.5±4.6	39.8±5.3	<0.001
E/A ratio	0.79±0.24	0.81±0.38	0.56

Data are expressed as mean±SD, median and inter-quartile range or as percent frequency, as appropriate.

Follow-up study

After the initial assessment patients were followed up for 39±19 months (range 0.8-62.0 months). During the follow-up fatal CV events [myocardial and mesenteric infarction, heart failure, ECG documented arrhythmia, sudden death, major venous and arterial thromboses (excluded AV fistula thromboses), peripheral vascular disease, pulmonary embolism and stroke] and death were accurately recorded. Each death was reviewed and assigned an underlying cause by a panel of 5 physicians. As a part of the review process, all available medical information about each death was collected. This information always included study and hospitalization records. In the case of an out-of-hospital death family members were interviewed by telephone to better ascertain the circumstances surrounding death.

Laboratory measurements

To minimize the effect of cyclic changes in extra-cellular volume all studies in hemodialysis patients were performed between 8.00 A.M. and 10.00 A.M. midweek, during the dialysis

interval. Sampling was performed at empty abdomen in CAPD patients. After 20-30 min of quiet resting in semi-recumbent position samples were taken into chilled EDTA vacutainers, placed immediately on ice, centrifuged within 30 min at – 4°C and the plasma stored at -80°C before assay. Serum lipids, albumin, calcium and phosphate and hemoglobin measurements were made by standard methods in the routine clinical laboratory. Methods of measurements of serum C-Reactive Protein (CRP) and plasma total homocysteine were detailed in a previous paper[13].

Echocardiography

For hemodialysis patients these studies were performed in a non-dialysis day and for CAPD patients at empty abdomen (always within 2 hours after blood sampling). Left ventricular mass (LVM) was calculated according to the Devereux formula and indexed to height$^{2.7}$ (LVMI)[2]. Left ventricular hypertrophy (LVH) was defined by a LVMI of over 47 g/m$^{2.7}$ in women or over 50 g/m$^{2.7}$ in men. Values indicative of concentric and eccentric left ventricular geometry were established on the basis of an age-specific reference standard[14]. Mitral inflow was assessed with pulsed-wave Doppler echocardiography from the apical 4-chamber view. Left atrial diameter was taken in the parasternal long axis view in M-mode at end-systole. LAV was calculated by the biplane area-length method from apical 4-chamber and 2-chamber view and measurements were obtained at the end of left ventricle systole[15]. These measurements were indexed both for BSA[15] and for height powered to 2.7, as proposed by De Simone for LV mass[16]. From the mitral inflow profile the ratio of early (E) to late atrial (A) mitral Doppler peak flow velocity (E/A ratio) was calculated ad an index of LV diastolic function. A value of LVEF <50% was considered indicative of LV systolic dysfunction[4].

Statistical Analysis

Data are reported as mean ±± SD, median and inter-quartile range or as percent frequency and comparisons between two groups were made by T-Test, Mann-Whitney Test or Chi Squared Test, as appropriate. Comparisons among more than two groups were made by One Way ANOVA. The relationship between paired variables was analysed by Pearson product moment correlation coefficient. Variables that shown a positively skewed distribution were log transformed (lg_{10}) before the correlation study. The independent predictors of LAV were identified by multiple regression analysis including all univariate predictors of LAV.

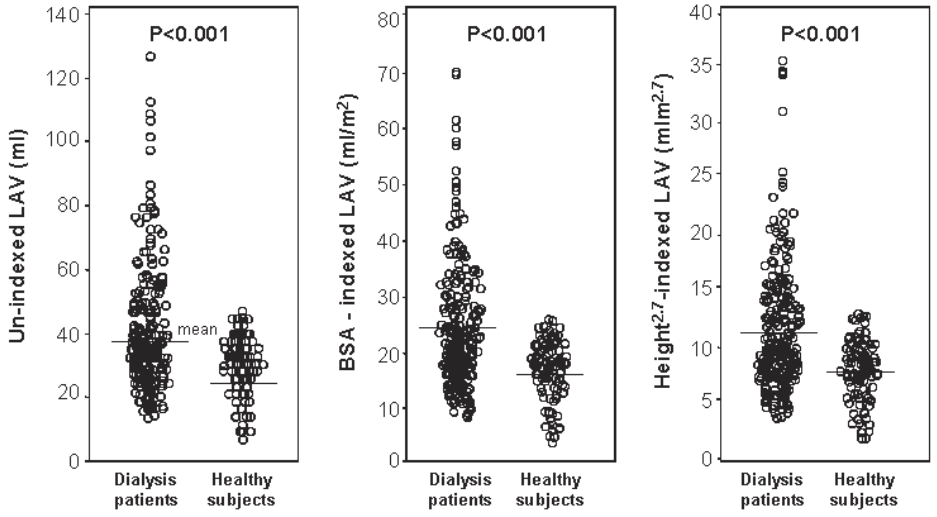
The independent prognostic value of LAV (either in terms of un-indexed values or as indexed by BSA and height$^{2.7}$) for all cause mortality was analysed by multiple Cox regression analysis. To construct multivariate Cox's models, we considered a series of traditional risk factors (age, sex, smoking, diabetes, serum cholesterol, arterial pressure, heart rate and anti-hypertensive treatment) and factors peculiar to ESRD [treatment modality (HD/CAPD), duration of RDT, haemoglobin, serum albumin, serum calcium and phosphate, serum CRP and plasma total homocysteine]. In a first step we identified variables that were associated

(with P<0.05) to all-cause mortality (see Table 1). These variables were then jointly included into a multivariate Cox model (basic model), always including also the treatment modality (HD/CAPD). We then tested by the -2 Log Likelihood statistic[17] whether the addition of LAV (either un-indexed or indexed) added significant prognostic information to the basic model. This procedure compares different models fitted to the same set of survival data and the smaller the -2 Log L value, the better the agreement between the model and the observed data. The difference between the - 2 Log L of the models, which are being compared, gives a statistical estimate as to which of them provides a better fit to the data. Furthermore, to test whether LAV (either un-indexed or indexed for BSA and height$^{2.7}$) had a predictive value for all cause death beyond and above that provided by LV mass and function we analyzed its prognostic power in Cox models including LVMI and LVEF. Hazard ratios (HR) and their 95% confidence intervals (CI) were calculated with the use of the estimated regression coefficients and their standard errors in the Cox regression analysis. All calculations were done using a standard statistical package (SPSS for Windows Version 9.0.1, 11 Mar-1999, Chicago, Illinois, USA).

RESULTS

Both un-indexed and indexed LAV resulted to be significantly higher (P<0.001) in dialysis patients than in healthy subjects (**Fig. 1**). The proportion of patients with enlarged LAV (i.e. LAV > 95th percentile of the distribution in healthy subjects) were 28% with the un-indexed estimate and 35% and 30% with the BSA and height$^{2.7}$ adjusted estimates. BSA and height$^{2.7}$

Figure 1. Un-indexed and indexed LAV in dialysis patients and in healthy subjects.



indexed LAV tended to be higher (P=0.06 and P=0.13) in the 34 patients with mild to moderate valvular heart disease (26.6 ± 9.1 ml/m^2 and 12 ± 3.9 ml/m$^{2.7}$) than in the 215 patients with normal heart valves (23.2 ± 11.3 ml/m^2 and 10.8 ± 5.6 ml/m$^{2.7}$). The exclusion of patients with valvular heart disease did not modify the difference between ESRD patients and healthy subjects (P<0.001).

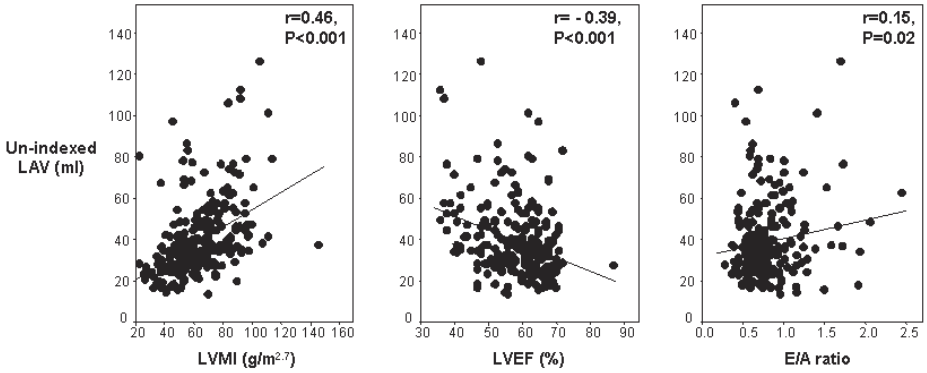
Correlates of LAV

Un-indexed LAV was related directly with age (0.17, P=0.007), male sex (r=0.17, P=0.009), smoking (r=0.17, P=0.007), systolic and pulse pressure (both r=0.16, P=0.01), anti-hypertensive therapy (r=0.26, P<0.001) and previous CV events (r=0.21, P=0.001), and inversely with haemoglobin (r= - 0.13, P=0.03) and albumin (r= - 0.32, P<0.001). No association was found between un-indexed LAV and BMI (r=0.09, P=0.17). Furthermore, un-indexed estimates were also related directly with LVMI and E/A ratio and inversely with LVEF (Fig. 2). On multiple regression analysis (Table 2) only LVMI, LVEF, E/A ratio and anti-hypertensive treatment retained an independent link with LAV. LAV indexed either by BSA or by height$^{2.7}$ displayed univariate and multivariate relationships with the above mentioned risk factors similar to those of un-indexed LAV (data not shown).

Left atrial volume, LV mass, LV geometry and function

One hundred and ninety-three (77.5%) patients displayed LVH that was concentric in 97 cases and eccentric in 96 cases. Sixteen patients had cardiac remodelling (6.4%) and 40 had normal LV mass and geometry (16.1%). Systolic and diastolic dysfunction were present in 53 (21.3%) and in 205 (82.3%) patients, respectively. Data analysis according to left ventricular geometry revealed that both un-indexed and indexed LAV were low in patients without alterations in LVM and geometry, intermediate in patients with LV remodeling and similarly increased in those with concentric and eccentric LVH (Fig. 3).

Figure 2. Relationship between un-indexed LAV with left ventricular mass index (LVMI), left ventricular ejection fraction (LFEF) and E/A ratio.



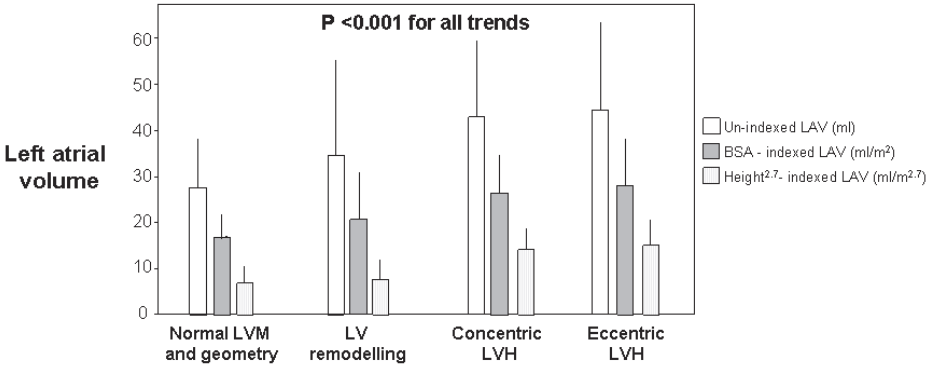
Data are correlation coefficients (r) and P values.

Table 2 Multiple Regression Models of un-indexed left atrial volume (LAV)
Dependent Variable: Un-indexed LAV

	β	P
LVMI	**0.24**	**0.001**
Ejection Fraction	**- 0.19**	**0.004**
Anti-hypertensive treatment	**0.15**	**0.02**
E/A ratio	**0.14**	**0.01**
Treatment modality (0=HD; 1=CAPD)	0.12	0.08
Previous CV events	0.11	0.08
Sex	0.09	0.18
Haemoglobin	-0.06	0.27
Age	0.04	0.54
Pulse Pressure	-0.04	0.72
Smoking	0.01	0.83
Albumin	0.01	0.84
Systolic Pressure	-0.005	0.97

Data are reported as standardised regression coefficients (β) and P values.

Figure 3. Association between un-indexed and indexed LAV with left ventricular geometry.



Data are expressed as mean ± SD and comparisons among groups were made by One Way ANOVA.

Prospective Cohort Study :Prognostic value of un-indexed and indexed LAV

During the follow-up period 113 patients died, 71 of them (i.e. 63% of total deaths) of CV causes (Table 3). Patients who died during the follow up were older, with a greater proportion of males, diabetics, smokers and with previous CV events than survivors. Furthermore, those who died had higher pulse pressure and serum CRP and lower serum albumin than survivors. Death was also associated with worse echocardiographic parameters of LV mass, left atrium and systolic function ($P \leq 0.001$)(Table 1). The exclusion of patients with valvular heart disease did not change the difference in LAV between non-survivors and survivors ($P<0.001$). Un-indexed LAV added significant prognostic power to multivariate Cox model of all-cause death and resulted to be an independent predictor of this outcome (Table 4). The indexation of LAV for height$^{2.7}$ provided significantly stronger prognostic value in comparison to unadjusted and BSA-adjusted LAV (Fig. 4). The prognostic power of height-indexed LAV

Table 3 Causes of death in the study cohort.

Causes of death	n
Cardiovascular	
Stroke	19
Heart Failure	14
Myocardial Infarction	12
Mesenteric Infarction	5
Arrhythmia	5
Sudden Death	11
Pulmonary Embolism	3
Peripheral Vascular Disease	2
Other causes	
Cachexia	12
Sepsis/Infection	17
Neoplasia	4
Hyperkalemia	4
Gastrointestinal haemorrage	2
Chronic Obstructive Pulmonary Disease	1
Haemoptysis	1
Treatment withdrawal	1
Total	113

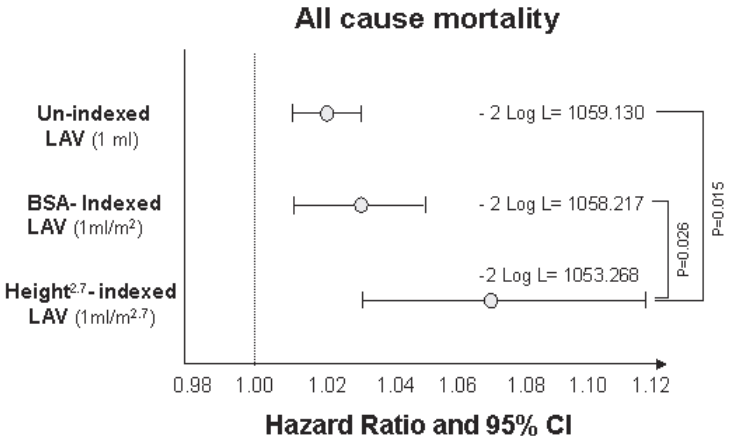
Table 4 Multiple Cox regression models of un-indexed left atrial volume for all cause mortality.

Covariates	Units of increase	Hazard Ratio and 95% CI	P
Age	1 year	1.05(1.03-1.07)	<0.001
Male sex		1.26(0.75-2.13)	0.38
Smoking		1.33(0.80-2.22)	0.27
Diabetes		1.49(0.93-2.40)	0.10
Previous CV events		1.95(1.30-2.93)	0.001
Diastolic Pressure	1 mmHg	0.99(0.98-1.01)	0.43
Pulse Pressure	1 mmHg	1.00(0.99-1.01)	0.70
Albumin	1 g/L	0.96(0.92-1.00)	0.05
CRP	10 mg/L	1.07(1.01-1.15)	0.04
Treatment modality	0=HD; 1=CAPD	0.78(0.44-1.37)	0.38
Un-indexed LAV	1 ml	1.02(1.01-1.03)	0.007

Data are reported as hazard ratio and 95% CI.

for total mortality was maintained also when patients with valvular heart disease were excluded from the analysis [HR (1 ml/$m^{2.7}$): 1.09, 95% CI: 1.05-1.14, P<0.001]. Notably, LA volume indexed by height$^{2.7}$ [HR (1 ml/$m^{2.7}$): 1.05, 95% CI: 1.01-1.09, P=0.03] maintained an independent predictive value for all cause death also when LVMI and LVEF were jointly forced into the Cox's model. Neither crude (P=0.12) nor BSA adjusted (P=0.11) LAV had an independent association with death when tested into the Cox model including LVMI and LVEF.

Figure 4. Hazard ratios and 95% CIs for all-cause mortality associated with LAV either un-indexed or indexed by BSA or height$^{2.7}$**.**



Data were appropriately adjusted for covariates listed in Table 4. In Figure we also reported the -2 Log Likelihood test. As shown, LAV as indexed by height$^{2.7}$ gives the best data fitting with the observed data because it provides the smaller -2 Log L value in comparison to the un-indexed (P=0.015) or BSA indexed (P=0.026) LAV.

DISCUSSION

This study shows that the volume of the left atrium is markedly increased in patients with ESRD and independently associated with LVH and with LV systolic and diastolic dysfunction. Furthermore, height$^{2.7}$- indexed LAV adds independent predictive value for mortality over and beyond LV mass and function and other risk factors in ESRD patients.

LA size is currently estimated either on the basis of LA diameter or biplane volume measurement. We focused on the biplane volume estimate because this method is considered as the best to determine left atrial size in clinical research and in clinical practice as well [15;18]. Left atrial dimensions are much dependent on left ventricular pressure and when the ventricle is exposed to pressure and/or volume overload such as in hypertension or aortic valve disease, this mechanism results in a progressive left atrial enlargement [19;20]. The magnitude of this change depends on the duration of pressure/volume alterations and on left ventricular diastolic function. Patients with ESRD are notoriously volume expanded and with systemic hypertension. Furthermore, as previously alluded, alterations in LV mass and function are pervasive in these patients[1;4]. These structural and functional disturbances were indeed all associated with LA enlargement in our population, particularly so left ventricular mass and systolic function (Fig.2). Measurements of the left atrium have been rarely performed in dialysis patients. In general LA size was used to estimate volume overload and/or dialysis induced change in blood volume [21-23]. Our observations confirming the substantial dependence of atrial volume on structural and functional parameters of LV mass and function cast

doubts on the reliability and the usefulness of these estimates because LV mass, ejection fraction (an indicator of systolic function) and the E/A ratio (an indicator of diastolic function) were all independently related to LAV thus making unlikely that this measurement be a reliable indicator of the volume status in these high risk patients. This likely applies not only in steady state conditions, as suggested by the present data, but also during acute changes in extracellular volume. Fluid subtraction during dialysis improves mechanical functions of left atrium [24] and induces a marked change in Doppler mitral early diastolic velocity[11] which may affect to an important extent the relationship between extracellular volume and atrial size. Indeed the change in LAV during volume unloading in these patients is much less than that of mitral early diastolic velocity[11].

Left atrial size is influenced not only by LV mass and function but also by heart geometry. In essential hypertensives at low CV risk LA volume was higher in patients with concentric LVH than in those with eccentric LVH [9;19]. In contrast an opposite association emerged in high risk hypertensive patients[25]. In the present study, in ESRD patients, atrial dilatation was of similar degree in patients with concentric and eccentric LVH. The similar atrial enlargement in ESRD patients with concentric and eccentric LVH likely depends on the fact that volume overload is a much more frequent hemodynamic component of hypertension in dialysis patients than in essential hypertensives at low-medium risk. Like in previous studies we found that mild to moderate valvular heart disease is relatively common in the dialysis population. Severe valve disease is rare in the dialysis population (15 to 19 cases per 10.000 patients)[26] and no such case was detected in our cohort. Yet, the left atrium tended to be enlarged in patients with mild to moderate valvular involvement. Thus, as it is the case in high risk hypertensive patients[25], also in dialysis patients an enlarged atrium is a sign that should prompt a careful examination of the valvular apparatus.

The main finding in the present study is the demonstration that indexation of LA volume by height improves substantially the prediction power for death in ESRD patients. Atrial dilatation promotes stasis of blood thus favouring thrombogenesis and embolization[27]. Left atrial size is directly related with LV mass[28], an observation we specifically confirmed in ESRD patients, and it is therefore possible that the predictive power of LA size just depends on the fact that this measurement reflects LV mass. Indeed, like in the Framingham heart study[7] we found that the predictive power for death of un-indexed and BSA-indexed LA volume became non significant after the inclusion of LV mass into the predictive model. Yet height-indexed LA volume retained an independent predictive power for this outcome. We have previously shown that appropriately indexing LV mass is of particular importance in ESRD and on theoretical grounds the same advantage should also apply to atrial volume[29]. Malnutrition is very frequent in patients undergoing dialysis, and body weight is substantially reduced at all height groupings in these patients in comparison to normal individuals[30] which may lead to an overestimation of LAV when cardiac measurements are indexed by BSA. On the other hand, expanded extracellular volume (which increases body weight) may produce

an underestimate of LAV because augments the denominator (i.e. BSA). Thus, the estimation of LAV may be distorted in an unpredictable way and in opposite directions by malnutrition and extracellular volume expansion when the BSA index is used in dialysis patients. The present data extend to LAV the importance of the indexation by height proposed by the De Simone for LV mass [16;29], at least in ESRD patients. Our study was not powerful enough to allow detailed analyses to identify the subgroup of events specifically affected by LAV. As alluded to before, LA enlargement favours thrombus formation, a process already facilitated by hyperfibrinogenemia[31] and by thrombogenic alterations in ESRD. Although not apparent in the present study, an enlarged left atrium predisposes to arrhythmias and in particular to atrial fibrillation[7;8], a frequent problem which predicts death in these patients[32;33]. On the other hand, it is also possible that LAV just captures residual confounding attributable to the imprecise measurement of LV mass and function by echocardiography or to other unmeasured risk factors.

In conclusion LAV is markedly increased in patients with ESRD and it is independently associated with LV mass and function. LAV indexed by height$^{2.7}$ provides prognostic information above and beyond LV mass, LV ejection fraction and other risk factors and represents therefore an additional echocardiographic parameter useful for risk stratification in the ESRD population.

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Chapter 4.2

Left Atrial Volume Monitoring and Cardiovascular Risk in End Stage Renal Disease: A Prospective Cohort Study.

ABSTRACT

Left atrial volume (LAV), as indexed by height$^{2.7}$, has recently emerged as an useful echocardiographic measurement to refine the estimate of cardiovascular (CV) risk in end stage renal disease (ESRD). Whether progression or regression in LAV has prognostic value in ESRD patients is still unknown.

We tested the prognostic value for CV events of changes in LAV in a cohort of 191 dialysis patients. Echocardiography was performed twice, 17±2 months apart. Changes in LAV occurring between the second and the first echocardiographic study were used to predict CV events during the ensuing 27±13 months.

During the follow-up there was a significant increase in LAV (from 10.5±5.0 ml/m$^{2.7}$ to 11.6±5.6 ml/m$^{2.7}$, P<0.001). After the second echocardiographic study, 76 patients died [52 of them (i.e. 68%) of CV causes] and 33 had non fatal CV events. The independent association between changes in LAV and CV events was analysed in a multiple Cox regression model taking into account a series of potential confounders including baseline LAV and left ventricular mass (LVMI) and geometry. In these models a 1 ml/m$^{2.7}$/yr increase in LAV was associated with a 12% increase in the relative risk of fatal and non fatal CV events (P<0.001).

Changes in LAV predict incident CV events in dialysis patients independently of the corresponding baseline measurement and of LVMI. Monitoring LA size by echocardiography is useful for monitoring CV risk in ESRD patients.

Key words: cardiovascular risk, dialysis**,** echocardiography, left atrial volume.

INTRODUCTION

Echocardiography is now an established technique to estimate the risk of cardiovascular (CV) complications and to guide treatment in patients with end-stage renal disease (ESRD) (1-4). The left atrium is an important potential risk marker because it fulfils three major physiologic roles that impact upon left ventricular (LV) filling and performance. Indeed, it acts as a contractile pump, as a reservoir that collects pulmonary venous return and as a conduit for the passage of stored blood from the left atrium to the left ventricle during early ventricular diastole. Although no specific recommendation can be extrapolated to ESRD, current guidelines in the general population jointly issued by the American College of Cardiology, the American Heart Association and the European Society of Cardiology (5) consider the measurement of left atrial volume (LAV) as clinically relevant information and emphasize that an increased LAV is associated with adverse CV outcomes (6-8).

Left ventricular mass index (LVMI) (3,9) and indicators of LV contractility such as ejection fraction and midwall fractional shortening (mwFS) (10) as well as LV chamber volume (11) provide valuable information for initial risk stratification and for risk monitoring in the follow up of dialysis patients. We have recently reported that, independently of these indicators, the volume of the left atrium, as indexed by height$^{2.7}$, may be useful to refine the risk estimate specifically in ESRD (12). Indeed the prognostic power of LAV in this population is of degree comparable to that of LVMI and, when tested in models including both measurements, it maintains an independent relationship with incident CV events (12). As alluded to before, serial measurements of both LVMI and mwFS provide prognostic information beyond that given by single studies of these echocardiographic measurements (13-14). In other words, progression or regression of LVH and LV dysfunction herald changes in the risk of incident cardiovascular events and for this reason repeated echocardiographic studies are considered useful to monitor the evolution of cardiomyopathy in these patients. Whether progression or regression in LAV has prognostic value in ESRD patients is still unknown. The issue is of relevance because extra-cellular volume overload, valvular heart disease and alterations in LV mass and compliance, all factors impinging upon LAV, are common in the dialysis population and because at least some of these factors may be modified by dialysis as well as by drug treatment. Thus investigating whether or not changes in LAV reflect changes in the risk of death and cardiovascular complications and whether these associations are independent of ongoing changes in LV mass and function is an important question which may have clinical implications.

In this prospective cohort study we examined the prognostic power of serial measurements of LAV in dialysis patients who attended the baseline and follow up echocardiographic measurements in the CREED study (Cardiovascular Risk Extended Evaluation in Dialysis patients: a multicenter cohort study aimed at establishing the role of traditional and emerging risk factors in the high CV risk of ESRD patients). To this aim we related changes in

LAV to all-cause mortality and incident CV events and tested whether these relationships are independent of baseline LAV, LVMI, LV systolic function, previous cardiovascular events and of a series of traditional and non traditional risk factors in ESRD patients.

METHODS

Protocol

The protocol was in conformity to the Declaration of Helsinki and informed consent was obtained from each participant. All studies were performed between 8 A.M. and 1 P.M.

Original study cohort

The original dialysis cohort was formed by two hundred and eighty-three patients (158 males and 125 females). At enrolment these patients had been on regular dialysis treatment (RDT) for at least 6 months (median duration of RDT: 41 months, inter-quartile range 18-93 months). The enrolment criteria in this cohort were no history of congestive heart failure [defined as dyspnea in addition to two of the following conditions - raised jugular pressure, bibasilar crackles, pulmonary venous hypertension or interstitial edema on chest X ray, requiring hospitalization or extra ultra-ultrafiltration (15)], LV ejection fraction $\geq$35% and no inter-current or terminal illnesses. Thirty-four patients were excluded because of low quality echocardiographic left atrial volume recordings. Thus 249 patients (142 males and 107 females) entered into this study. The main demographic and clinical characteristics of the cohort are detailed in **Table 1**. Hemodialysis patients were being treated thrice weekly with standard bicarbonate dialysis (Na 138 mmol/L, HCO_3 35 mmol/L, K 1.5 mmol/L, Ca 1.25 mmol/L, Mg 0.75 mmol/L) and cuprophan or semi-synthetic membranes (dialysis filters surface area: 1.1-1.7 m^2). The average Kt/V in these patients was 1.22±0.27. Chronic ambulatory peritoneal dialysis (CAPD) patients (n=50) were all on 4 exchanges/day schedule with standard dialysis bags. The average weekly Kt/V in these patients was 1.66±0.32. One hundred and thirty patients were on treatment with erythropoietin. One hundred and ten patients were being treated with anti-hypertensive drugs (77 on mono-therapy with ACE inhibitors, AT-1 antagonists, calcium channel blockers, alpha and beta-blockers and 33 on double or triple therapy with various combinations of these drugs).

Patients who repeated the echocardiographic study

Thirty-six patients out of 249 who entered into this study died before the time at which the second echocardiographic study was performed, 5 patients underwent renal transplantation and 17 patients could not repeat echocardiography for logistic reasons. Therefore 191 patients were left for this study aimed at defining the prognostic value of changes in left atrial volume (see Table 1). No patient had severe valvular heart disease. However 23 patients

Table 1 Demographic, somatometric, clinical, biochemical and echocardiographic data of patients in the original study cohort and in those who repeated the echocardiographic evaluation of LAV.

	Original cohort (n=249)	*Patients who repeated echocardiography (n=191)*		***P*** *(second vs first visit)*
		First visit	**Second visit**	
Age (years)	60±15	59.2±15.5	60.7±15.5	**<0.001**
Males n. (%)	142 (57%)	110(58%)	110(58%)	1.00
Diabetics n. (%)	35 (14%)	26(14%)	26(14%)	1.00
Smokers n. (%)	107 (43%)	75(39%)	75(39%)	1.00
Patients on anti-hypertensive therapy n. (%)	110 (44%)	81(42%)	74 (39%)	0.13
Systolic pressure (mmHg)	133±22	132.5±22.5	130.8±24.4	0.15
Diastolic pressure (mmHg)	75±12	75.1±13.0	73.1±13.1	**0.008**
Heart rate (beats/min)	81±12	80.9±12.1	80.8±9.8	0.99
Hemoglobin (g/L)	106±19	104.9±17.9	108.3±15.9	**0.02**
Albumin (g/L)	40±6	40.4±5.6	35.7±5.2	**<0.001**
Cholesterol (mg/dL)	206±54	175.8±45.7	202.4±54.0	**<0.001**
Calcium * Phosphate ($mMol^2/L^2$)	4.45±1.17	4.46±1.17	4.22±1.18	**0.007**
C-Reactive Protein (mg/L)	7.3 (3.4-16.3)	7.3 (3.4-16.0)	NA	…
Homocysteine (µMol/L)	27.0 (19.6-41.1)	25.5 (19.2-38.4)	NA	…
LAV (ml)	38.6±17.9	38.0±17.6	42.1±20.8	**<0.001**
Midwall fractional shortening (%)	14.1±3.3	14.4±3.3	14.0±2.8	0.07
LAV ($height^{2.7}$ indexed) ($ml/m^{2.7}$)	10.7±5.4	10.5±5.0	11.6±5.6	**<0.001**
E/A ratio	0.80±0.31	0.80±0.29	0.79±0.30	0.87
LVMI ($g/m^{2.7}$)	64.1±19.9	62.1±18.5	66.8±20.4	**<0.001**

NA: Not available

had mild to moderate valvular heart disease: 2 patients had combined valvular stenosis and insufficiency (mitral in 1 case and aortic in 1 case); 5 patients had isolated valvular stenosis (mitral in 1 case and aortic in 4 cases) and 16 patients had isolated valvular insufficiency (mitral in 10 cases, aortic in 5 cases and a simultaneous involvement of two valves in 1 case).

Follow up

After the initial assessment patients were followed up by the nephrologists participating into the study. The study was purely observational and therefore it did not contemplate changes in treatment policy. The second echocardiographic study was performed from 11 to 23 months (average 17 months) after the baseline study. The overall duration of the follow up was 45±13 months. The duration of follow up after the second echocardiographic study was 27±13 months. Since in the present study we were interested in establishing the prognostic value of changes in left atrial volume, all survival analyses reported herein apply to the follow up after the second echocardiographic study (see Statistical Analysis).

End-point evaluation

During the follow-up cardiovascular events (ECG documented anginal episodes and myocardial infarction, heart failure, ECG documented arrhythmia, transient ischemic attacks, stroke and other thrombotic events except arterio-venous fistula thromboses) and death were accurately recorded. Each death was reviewed and assigned an underlying cause by a panel of 5 physicians. As a part of the review process, all available medical information about each death was collected. This information always included study and hospitalization records. In the case of an out-of-hospital death family members were interviewed by telephone to better ascertain the circumstances surrounding death. As alluded to before, for the purpose of establishing the prognostic value of progression in systolic dysfunction only events (death and cardiovascular events) occurring after the second echocardiogram were considered.

Echocardiography

These studies were performed mid-week in a non-dialysis day for hemodialysis patients and at empty abdomen for CAPD patients. At the time of the echocardiographic examination, investigators involved in echocardiographic studies were unaware of patients' clinical data. Left ventricular mass (LVM) was calculated according to the Devereux formula and indexed to height$^{2.7}$ (LVMI), as proposed by De Simone [16].The relative wall thickness (RWT: 2*posterior wall thickness/left ventricular end diastolic diameter) was also calculated, as an index of the LV geometric pattern. Analysis of LV geometry was done according to Ganau et al. (17). Mitral inflow was assessed with pulsed-wave Doppler echocardiography from the apical 4-chamber view. Left atrial volume was calculated by the biplane method of discs (18) at the end of left ventricle systole. LAV data were analyzed as height$^{2.7}$ indexed estimates because this indexation provides the best prognostic power in dialysis patients (9). From the mitral inflow profile the ratio of early (E) to late atrial (A) mitral Doppler peak flow velocity (E/A ratio) was calculated ad an index of LV diastolic function. Mid-wall fractional shortening (mwFS), which is a reliable indicator of LV performance (inter-observer reproducibility: 4.0%; intra-observer reproducibility: 4.5%) (19), was calculated according to the method of Shimizu et al (20) as described in full detail by De Simone (21). Changes in LAV were quantified by subtracting LAV at the second study from that obtained at baseline study and by factoring this difference for the time interval between the two studies.

Biochemical Measurements

Blood sampling for the measurement of routine and special biochemical measurements were performed before echocardiographic studies. The methods used for the determination of serum C-Reactive Protein (CRP) and plasma total homocysteine were detailed in a previous publication (22).

Statistical Analysis

Data are expressed as mean ±± SD (normally distributed data), median and inter-quartile range (non normally distributed data) or as percent frequencies and within subjects comparisons were made by paired t-test and Chi Squared test, as appropriate. The relationship between paired variables was analyzed by Pearson product moment correlation coefficient.

The association between changes in LAV and all-cause death and fatal and non fatal CV events was analyzed by Kaplan-Meier analysis and by multivariate Cox's proportional hazards model. The following covariates were initially considered in the Cox survival analysis to produce a final, parsimonious model: changes in LAV and baseline LAV, changes in E/A and the corresponding baseline value, baseline LV mass and function and their changes during the follow-up, treatment modality, age, sex, diabetes and inter-dialysis weight gain; baseline systolic pressure and heart rate and their changes from baseline to the follow up visit; previous CV events and anti-hypertensive therapy; smoking, serum cholesterol, haemoglobin, albumin, Ca x P product and the change in these covariates at follow up. Furthermore we also tested two emerging risk factors (CRP and Homocysteine) which were available at baseline visit only. Variables with a $P \leq 0.10$ were retained into the final model in which we always forced the treatment modality. By this approach we constructed models of adequate statistical power (at least 10 events for each variable in the final model). To assess the functional form of LVMI into the survival analysis [i.e. LVMI dichotomised according to the mean, the median and the 75th percentile of the relative data distribution] we used the Martingale residuals analysis (23). By this analysis we found that the "75th percentile" was the most appropriate functional form of LVMI to be considered for the stratified analysis reported in Fig. 2.

All calculations were made using a standard statistical package (SPSS for Windows Version 9.0.1, Chicago, Illinois, USA, 11 Mar-1999).

RESULTS

Demographic, anthropometric, clinical and biochemical data of the original study cohort and of patients who repeated the echocardiographic study are summarized in Table 1. Between the first and the second echocardiographic study, diastolic pressure, serum albumin and calcium*phosphate product showed a significant decrease while haemoglobin and serum cholesterol increased (+ 3% and + 15%, respectively). Overall, there was a highly significant increase in both LAV and LV mass (Table 1) while mwFS showed an opposite trend. On univariate analysis, changes in LAV were directly related to changes in LV mass (r= 0.18, P=0.01) but unrelated to changes in mwFS (r=-0.07, P=0.34). The distribution of LAV changes in the whole study cohort is described in Fig. 1.

Figure 1. Distribution of LAV changes in the study cohort. Data are expressed ml/m$^{2.7}$/year.

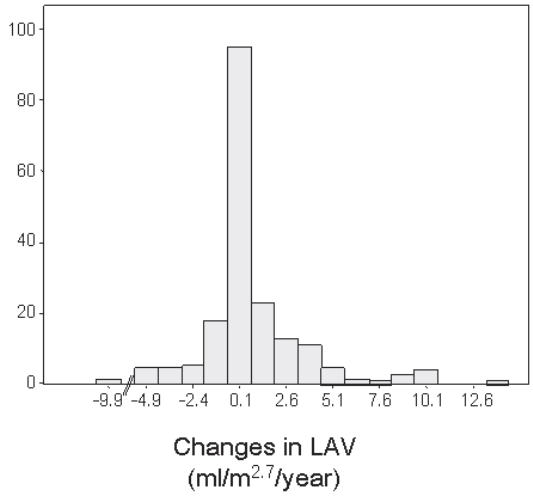
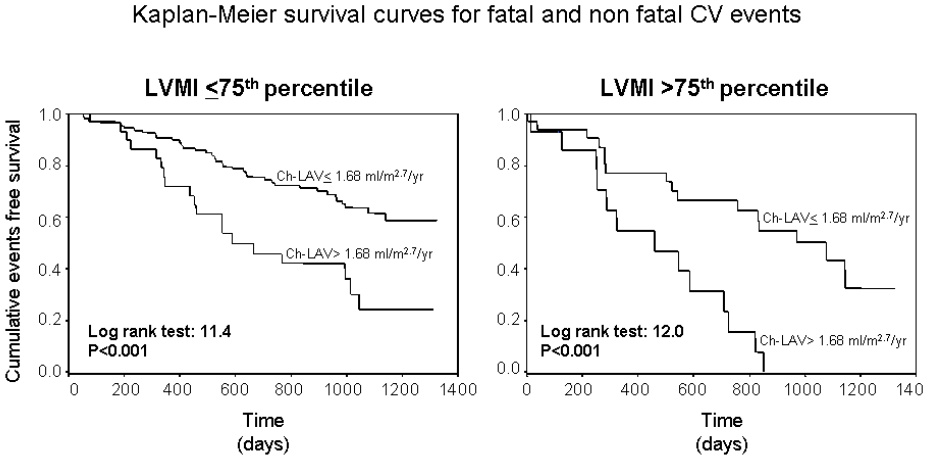


Figure 2. Stratified Kaplan-Meier analysis for fatal and non fatal cardiovascular events of changes in LAV in patients below and above the 75th percentile of left ventricular mass.



To assess the functional form of LVMI better related to survival analysis [i.e. LVMI dichotomised according to the mean, the median and the 75th percentile of the relative data distribution] we used the Martingale residuals analysis (23). By this analysis we found that the "75th percentile" was the most appropriate functional form of LVMI to be considered for the stratified analysis reported in the figure.

Changes in LAV and incident fatal and non fatal CV events: univariate analysis

After the second echocardiographic study, 76 patients died [52 of them (i.e. 68%) of CV causes] and 33 had non fatal CV events. Changes in LAV did not differ in patients who died and in those who survived (P=0.64). On the other hand, the rate of increase in LAV was significantly higher (P<0.001) in patients with incident fatal and non fatal CV events (median: 0.59 ml/height$^{2.7}$/yr, inter-quartile range: 0.00-2.62 ml/height$^{2.7}$/yr) than in events free patients

(median: 0.11 ml/height$^{2.7}$/yr, inter-quartile range: -0.27-0.58 ml/height$^{2.7}$/yr) and in a Kaplan-Meier analysis patients with a relatively higher rate of increase in LAV (i.e. those with changes in LAV >75th percentile) had a relative risk of fatal and non fatal CV events that was about two times higher (hazard ratio: 1.85, 95% CI: 1.38-2.47) than that of the remaining patients (Log Rank Test: 23.1, P<0.001). In a Cox regression analysis changes in LV mass [HR (1 g/m$^{2.7}$/yr increase): 1.03, 95% CI: 1.01-1.05, P=0.03] and changes in mwFS [HR (1 %/yr increase): 0.86, 95% CI: 0.75-0.99, P=0.04], as adjusted for corresponding baseline values, resulted to be significantly associated to incident fatal and non fatal CV events. Of note, the rate of increase in LAV provided prognostic information for fatal and non fatal CV events beyond and above to that provided by LV mass because changes in LAV predicted CV outcomes also in an analysis stratified according to the 75th percentile of LVMI (Fig. 2).

Changes in LAV and incident fatal and non fatal CV events: multiple Cox regression analyses

The independent association between changes in LAV and fatal and non fatal CV events was analysed in a multiple Cox regression model taking into account a series of potential confounders including baseline LAV (to avoid the statistical phenomenon of the regression to the mean) and LV mass and geometry. In these models changes in LAV, as continuous variable or as dichotomized according to the 75th percentile of the corresponding data distribution, maintained an independent association, and a highly significant one, with incident CV outcomes (Table 2 a-b). Of note, in this analysis a 1 ml/m$^{2.7}$/yr increase in LAV was associated with a 12% increase in the relative risk of fatal and non fatal CV events. The association between changes in LAV and incident fatal and non fatal CV outcomes remained unmodified [HR (1 ml/m$^{2.7}$/yr increase in LAV): 1.12, 95% CI: 1.05-1.20, P<0.001] also after the inclusion of changes in LV mass and function into the Cox model.

Changes in LAV had an independent association with CV outcomes also when the crude (un-indexed) estimate of LAV was entered into the same model instead of the height$^{2.7}$ estimate [HR (1 ml/yr increase in un-indexed LAV): 1.03, 95% CI: 1.01-1.05, P=0.001].

DISCUSSION

This study shows that progressive increase in left atrial volume predicts incident CV events in dialysis patients independently of the corresponding baseline measurement of left atrial volume and of left ventricular mass and function. This finding indicates that monitoring LA size by echocardiography provides independent prognostic information in ESRD patients thus underscoring the relevance of repeated measurements of this echocardiographic measurement in the clinical management of these patients.

Table 2 Cox regression analysis of fatal and non fatal CV events.
a) Changes in LAV as continuous variable

	Units of increase	Hazard ratio and 95% CI	P
LAV	1 ml/$m^{2.7}$	1.07(1.01-1.13)	0.03
Changes in LAV*	1 ml/$m^{2.7}$/year	1.12(1.05-1.19)	<0.001
Age	1 year	1.03(1.01-1.05)	0.001
Smoking	0=no; 1=yes	1.89(1.20-2.98)	0.006
Haemoglobin	1 g/L	0.98(0.96-0.99)	0.04
Changes in Hemoglobin	1 g/L	0.98(0.96-0.99)	0.009
LV geometry	Normal LVMI	1**	
	Concentric LVH	1.29 (0.60-2.79)	0.51
	Eccentric LVH	2.00 (0.94-4.26)	0.07
Treatment modality	0=HD; 1=CAPD	0.72 (0.39-1.32)	0.29

* The hazard ratio (and the 95% confidence interval) associated to 1 SD increase in LAV changes (i.e. + 2.9 ml/$m^{2.7}$/year) was 1.38 (1.14-1.67) P<0.001.

b) Changes in LAV as dichotomised on the basis of 75th percentile of the relative data distribution

	Units of increase	Hazard ratio and 95% CI	P
LAV	1 ml/$m^{2.7}$	1.06 (0.99-1.12)	0.07
Changes in LAV			
≤75th percentile	≤1.68 ml/$m^{2.7}$/year	1**	
>75th percentile	>1.68 ml/$m^{2.7}$/year	2.99 (1.84-4.86)	<0.001
Age	1 year	1.03(1.01-1.05)	<0.001
Smoking	0=no; 1=yes	1.87(1.20-2.94)	0.006
Haemoglobin	1 g/L	0.98(0.96-0.99)	0.04
Changes in Hemoglobin	1 g/L	0.98(0.96-0.99)	0.02
LV geometry	Normal LVMI	1**	
	Concentric LVH	1.23(0.57-2.65)	0.60
	Eccentric LVH	2.24(1.05-4.77)	0.04
Treatment modality	0=HD; 1=CAPD	0.68(0.38-1.24)	0.21

****Reference group**
Forcing in Cox models heart rate, male sex, number of anti-hypertensive drugs and changes in LVMI [i.e. the univariate correlates of LAV changes (P≤0.10)], did not materially modify the strength of the association between fatal and non fatal CV events and changes in LAV either expressed as a continuous variable [hazard ratio (1 ml/$m^{2.7}$/year increase): 1.18, 95% CI: 1.07-1.31, P=0.001] or as dichotomised according to the threshold of 1.68 ml/$m^{2.7}$/year [hazard ratio: 2.64, 95% CI: 1.53-4.54, P<0.001].

LA enlargement is a hitherto overlooked component of the complex echocardiographic alterations observed in ESRD. In a recent study we found that LAV is substantially higher in dialysis patients than in age and sex matched healthy subjects and that LVH and systolic dysfunction are major correlates of left atrial size in this population

(12). Of note, we also found that LAV predicts incident CV events independently of traditional and non traditional risk factors and of LV mass and function (12). This finding indicates that the study of the left atrium may provide not only important anatomical details useful to interpret cardiomyopathy in ESRD but also complementary prognostic information for risk stratification in this condition. These observations are of relevance because populations with a high rate of CV complications like the ESRD population demand not only baseline risk strati-

fication but also periodic risk monitoring. To be useful for risk monitoring, clinical indicators should not only predict the outcome but be also sensitive to changes in risk which may occur over time as a consequence of disease progression or regression. Echocardiography-derived indicators provide important information for risk monitoring because worsening in LV mass and in LV systolic function predicts an increase in the risk of death and CV events. Thus, the issue whether changes in LAV convey independent prognostic information for risk monitoring is a relevant question that needs to be specifically tested with repeated echocardiographic studies adjusting for LV mass and function, i.e. the two major determinants of left atrial size in ESRD patients. In the present study we observed a 10 % increase in LAV in dialysis patients over a 15 months interval and changes in LAV were directly associated to changes in LV mass but unrelated to changes in mwFS. Of note, changes in LAV were proportionally higher than those in LV mass (+7.6%) and LV systolic function (-3.0%), indicating that left atrial volume expansion progresses at a higher rate than other established echocardiographic indicators of high risk. Our prospective observations show that the rate of increase in LAV was substantially higher in patients who developed CV events than in events free patients. Importantly, multivariate analysis indicated that changes in LAV had a prognostic power for CV outcomes beyond and above that provided by baseline LV mass and function and by their changes during the follow-up. Thus, LAV monitoring can be proposed as an adjunctive echocardiographic measurement which may refine risk monitoring in ESRD.

Two hypotheses can be made to explain the independent prognostic value of changes in LAV. 1) The first hypothesis rests on the several patho-physiological links between LAV and coronary heart disease and circulatory overload (24), i.e. links that may be even more pronounced in ESRD than in patients with other diseases. In other words, the predictive power of monitoring LAV may depend on the fact that this measurement conveys prognostic information attributable to the evolution of underlying coronary artery disease and volume expansion which are pervasive in ESRD. 2) The second hypothesis is methodological in nature. LAV is associated very strongly with LVH and with systolic dysfunction. In this perspective the additional prognostic value of changes in LAV may depend on the fact that this measurement captures residual confounding due to the relatively imprecise echocardiographic estimation of LV mass and function. Indeed LAV derived by the biplane method of discs (18) is more reproducible (i.e. more precise) than the most extensively used method quantifying LV mass, i.e. the M-Mode based measurement (25). LV mass estimated by standard M-Mode echocardiography is calculated on the basis of three variables (inter-ventricular septum thickness, posterior wall thickness and LV diameter) each of whom with a measurement error. In contrast LAV is estimated on the basis of an automatic computation based on LA contour reconstructing chamber volume which minimizes measurement error (18) .

Our study has limitations. Due to early mortality and censoring the cohort we considered in the follow-up study had a lower CV risk than the original cohort (survival cohort bias). This explains why solid predictors of death and CV events in patients with ESRD like CRP and

albumin failed to predict incident CV outcomes beyond the time of the second echocardiographic study. Yet we believe it is of importance that LAV changes maintain a strong predictive power for CV events in a relatively low risk cohort like the cohort of patients who survived beyond the second echocardiographic study. The second limitation is the study design. Ours is an observational study and for this reason the clinical usefulness of LAV monitoring remains to be assessed in interventional studies. Finally, new echocardiographic measurements allow LV mass and LAV be measured with considerable precision (26). Reassessment of the problem with this new methodology will certainly refine the relative role of left ventricular mass and left atrial volume in the risk assessment of patients with ESRD.

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Chapter 4.3

Traditional and non traditional risk factors as predictors of cerebro-vascular events in patients with end stage renal disease

ABSTRACT

End stage renal disease (ESRD) patients exhibit a higher risk of cerebro-vascular events as compared to the general population. In 283 ESRD patients followed-up for 10 years we investigated the long term predictive value for stroke and transient ischemic attacks (TIA) of traditional (Framingham) and non traditional risk factors. Data analysis was performed by a modified Cox's regression analysis for repeated events and by a competing-risks-analysis.

During the follow-up, 47 patients had at least one cerebro-vascular event (1 episode in 39 patients, 2 in 6 patients, 3 in 1 patient and 7 in 1 patient). Overall, 61 cerebro-vascular events occurred (5.1 events/100 person-years). On univariate Cox analysis, the risk of cerebro-vascular outcomes was directly related to age, smoking, diabetes, body mass index, systolic and pulse pressures, triglycerides, haemoglobin, history of stroke/TIA, history of arrhythmia and LV mass. Emerging risk factors in ESRD like norepinephrine, homocysteine, IL-6 and asymmetric dimethilarginine failed to predict these events. In a multiple Cox model including all univariate predictors of cerebro-vascular events only smoking, age, haemoglobin, pulse pressure and LVMI maintained an independent relationship with these outcomes. The direct link between haemoglobin and cerebro-vascular events was significantly stronger ($P<0.05$) than that of the same variable and all-cause death.

Multiple interventions aimed to reduce arterial stiffness, LV mass and smoking as well as to maintain haemoglobin within the recommended therapeutic range may have beneficial effects on the risk of cerebrovascular events in ESRD patients.

Key words: asymmetric dimethilarginine, cerebrovascular events, homocysteine, interleukin 6, norepinephrine.

INTRODUCTION

End stage renal disease (ESRD) patients exhibit a higher risk of cerebro-vascular events as compared to the general population (1) but risk factors identified so far do not fully account for the high incidence rate of these events in these patients (1-7). In the last decade, non-traditional risk factors such as anemia (8), inflammation (9), hyperhomocysteinemia (10), high sympathetic activity (11-12) and endothelial dysfunction as assessed by asymmetric dimethylarginine (ADMA) (13-14) have emerged as risk factors of paramount importance in ESRD but their predictive value for strokes and transient ischemic attacks (TIA) in this population is still unknown. Furthermore, left ventricular hypertrophy (LVH) is now considered as the strongest predictor of death in ESRD (15-16).

ESRD patients frequently suffer from recurrent cerebro-vascular events (17) but studies performed so far in the dialysis population focused only on the time to the first event analysis (4-7). Since population-based observations show that about 10% of individuals who experience an acute TIA or a minor stroke have a subsequent cerebro-vascular event within 3 months (18) and because only 1/3 of stroke episodes are fatal in ESRD patients (6), survival data analysis restricted to the first cerebro-vascular event may generate a significant loss of prognostic information.

We modelled the long term prognostic value for cerebro-vascular outcomes of traditional (Framingham) and non traditional risk factors by a modified Cox's regression analysis for repeated outcomes that allows to investigate multiple failure-time data of ordered events of the same type (19). Furthermore, because death from causes other than stroke precludes the occurrence of cerebro-vascular outcomes, the independent predictors of cerebro-vascular events in ESRD patients were also investigated by a competing risks model accounting for non stroke death (20).

METHODS

Protocol

The protocol was in conformity to the ethical guidelines of our Institutions and informed consent was obtained from each participant.

Study Cohort

Two-hundred and eighty-three ESRD patients [231 on haemodialysis and 52 on chronic ambulatory peritoneal dialysis (CAPD)] who had been on regular dialysis treatment (RDT) for at least 6 months with left ventricular ejection fraction (LVEF) >35% and without clinical evidence of heart failure were considered eligible for the Cardiovascular Risk Extended Evalu-

ation in Dialysis patients (CREED) study. All patients were virtually anuric (diuresis <200 ml/day).

Follow-up study

For the purpose of this study we considered fatal and non fatal cerebro-vascular events [stroke or transient ischemic attack (TIA)] and death occurring from January 1997 to August 2007. The diagnosis of stroke was made on the basis of computerized tomography, magnetic resonance imaging and/or clinical and neurological examination including patient's history and evaluation of symptoms surrounding the episode. Each event was reviewed and assigned an underlying cause by a panel of 5 physicians. TIA was defined according to the revision of the NIH classification document (21), as a sudden, focal neurological deficit lasting less than 24-hours and confined to an area of the brain or eye perfused by a specific artery. Neurological deficits include hemiparesis, hemiparesthesia, dysarthria, dysphasia, diplopia, peri-oral numbness, imbalance, and monocular blindness.

Laboratory measurements

Lipids, albumin, calcium, phosphate and haemoglobin were measured by using standard methods. Serum levels of interleukin-6 (IL-6) were measured by ELISA with the use of Quantikine High Sensitivity kits (R&D Systems Inc, Minneapolis, USA) (normal range <3.12 pg/mL). The plasma concentration of asymmetric dimethyl arginine (ADMA) was determined by high performance liquid chromatography (HPLC) (normal range<2.2 μMol/L). The plasma concentration of norepinephrine was measured by a commercially available RIA kit (Amicyl-test TM, Immunological Laboratories, Hamburg, Germany) (normal range<3.54 nMol/L). Plasma total homocysteine was determined by a HPLC method based on SBD-F (ammonium-7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate) fluorescence derivatization (normal range< 15 μMol/L).

Echocardiography

These studies were performed in a non-dialysis day for haemodialysis patients and at empty abdomen for CAPD patients within 2 hours after blood sampling. Left ventricular mass (LVM) was calculated according to the Devereux formula and indexed to height$^{2.7}$ (LVMI)(22). LVH was defined by a LVMI >47 g/m$^{2.7}$ in women or >50 g/m$^{2.7}$ in men. The height-based indexing of LVM minimises any potential distortion attributable to extra-cellular volume expansion (body surface area indexing being weight-sensitive) and provides more accurate prognostic information than that by body surface area in the dialysis population(15).

BP measurements

In haemodialysis patients pre- and post-dialysis blood pressures (BPs) were calculated as the average value of all recordings [12 measurements (i.e. 3/week)] taken during the month pre-

ceding the study. Because the reproducibility of the mean value of pre- and post- dialysis BPs approaches that of 24 hours Ambulatory Blood Pressure Monitoring better than that of pre- and post dialysis BPs(23), we considered this average value for global statistical assessment. In CAPD patients, BP values were obtained by averaging Home Blood Pressure Measurements (10 to 20 measurements/month).

Statistical analysis

Data are reported as mean ± SD, median and inter-quartile range (IQR) or as percent frequency, as appropriate.

Since more than one episode of cerebro-vascular events can occur in a single patient and because the occurrence of death from causes other than stroke precludes the occurrence of subsequent cerebro-vascular events, the independent prognostic factors for these outcomes was assessed by 1) a modified Cox's regression analysis using a conditional risk set method (*time from entry*) that allows to investigate multiple failure-time data of ordered events of the same type (19) and 2) by a competing risks model accounting for non stroke death (20).

In a first step, we performed unadjusted Cox regression analyses for cerebro-vascular outcomes by considering all risk factors listed in Table 1. Multiple Cox regression models were constructed by including all covariates which were related ($P \leq 0.05$) to cerebro-vascular events at univariate analysis. By this strategy we constructed multiple models of adequate statistical power (at least 1 variable every 5 events into the model). Data are expressed as hazard ratio (HR), 95% confidence interval (CI) and P value. All calculations were done using a standard statistical package (STATA 9*, StataCorp,* LP, TX, USA).

Study power

The study power was calculated by using a standard software (NCSS-PASS, Utah, USA). In previous studies in the CREED cohort over a shorter follow-up we found that IL-6(9), ADMA(24) and LV mass(15) provided 7.0%, 3.3% and 14.0% additional explanatory power, respectively, for cardiovascular events when added to Cox models including traditional risk factors. With this background in mind and by considering an expected cerebro-vascular event rate of 4.8 events/100 person-years (1) we calculated that a sample including at least 246 ESRD patients achieved 80% power to detect as significant (α-error=0.01) the associations between IL-6, homocysteine, norepinephrine, ADMA and LV mass and the incidence rate of cerebro-vascular outcomes.

Table 1 Characteristics of patients and unadjusted Cox regression analysis for cerebro-vascular events (conditional risk set approach) (19).

		Cox regression analysis(unadjusted)	
	Descriptive data	**Units of increase**	**Cerebrovascular events** (HR,95% CI and P)
Age(years)	61.0±15.4	1 year	**1.05(1.03-1.08),P<0.001**
Duration of dialysis (months)	41(18-93)	10 months	1.00(0.96-1.04),P=0.93
Treatment modality (CAPD/HD)	52/231	0=HD;1=CAPD	1.43(0.67-3.06),P=0.36
Male sex n.(%)	158 (55.8%)	0= female;1=male	0.86(0.48-1.53),P=0.60
Smokers n.(%)	117(41.3%)	0=no;1=yes	**2.51(1.42-4.44),P=0.002**
Diabetics n.(%)	43(15%)	0=no;1=yes	**2.13(1.08-4.18),P=0.03**
Body mass index(Kg/m^2)	24.9±4.4	1 kg/m^2	**1.05(1.00-1.11),P=0.05**
Systolic pressure(mmHg)	132.0±22.7	10 mmHg	**1.14(1.00-1.30),P=0.05**
Diastolic pressure(mmHg)	74.3±12.2	10 mmHg	0.89(0.68-1.16),P=0.38
Pulse pressure(mmHg)	57.7±16.5	10 mmHg	**1.35(1.16-1.58),P<0.001**
Heart rate(beats/min)	80.8±10.7	5 beats/min	0.92(0.67-1.27),P=0.60
On anti-hypertensive treatment n.(%)	117(41.3%)	0=untreated;1=treated	0.86(0.45-1.65),P=0.65
On treatment with erythropoietin n.(%)	149(52.7%)	0=no;1=yes	0.65(0.36-1.16),P=0.15
Cholesterol(mg/dL)	207.6±56.2	10 mg/dL	0.98(0.93-1.04),P=0.51
Triglycerides(mg/dL)	176.4±89.9	10 mg/dL	**1.03(1.01-1.06),P=0.003**
HDL cholesterol(mg/dL)	41.1±11.7	5 mg/dL	0.98(0.88-1.08),P=0.68
LDL cholesterol(mg/dL)	128.0±52.0	10 mg/dL	0.96(0.90-1.02),P=0.17
Haemoglobin(g/dL)	10.7±2.0	1 g/dL	**1.16(1.01-1.32),P=0.04**
Calcium*Phosphate(mMol^2/L^2)	4.43±1.13	1 mMol^2/L^2	1.08(0.89-1.31),P=0.44
Albumin(g/dL)	4.0±0.5	1 g/dL	0.76(0.46-1.25),P=0.27
Previous CV complications			
Myocardial infarction n.(%)	35(12.4%)	0=no;1=yes	1.53(0.70-3.34),P=0.28
Stroke/TIA n.(%)	42(15%)	0=no;1=yes	**2.72(1.40-5.28),P=0.003**
Angina pectoris n.(%)	82(29.0%)	0=no;1=yes	1.10(0.62-1.97),P=0.74
Arrhythmia n.(%)	22(7.8%)	0=no;1=yes	**3.09(1.27-7.50),P=0.01**
Peripheral vascular disease n.(%)	42(15%)	0=no;1=yes	1.86(0.87-3.97),P=0.11
LVMI(g/m$^{2.7}$)	64±20	1 g/m$^{2.7}$	**1.02 (1.01-1.04), P=0.005**
LVEF(%)	58±10	1 %	0.99(0.96-1.02), P=0.62
Norepinephrine(pg/mL)	561(314-993)	100 pg/mL	1.00 (0.95-1.06),P=0.91
Homocysteine(μMol/L)	27.3(19.9-41.2)	1 μMol/L	1.08(0.94-1.10),P=0.66
IL-6(pg/mL)	5.9(2.9-9.8)	3 pg/mL	1.00(0.96-1.03),P=0.84
ADMA(μMol/L)	3.03(1.73-4.08)	1 μMol/L	1.09(0.96-1.25),P=0.18

The relationship between each risk factor and cerebrovascular events is expressed as HR, 95% CI and P value.

RESULTS

Patients characteristics

The descriptive data of the study cohort are presented in Table 1. One-hundred and seventeen patients were habitual smokers (23±16 cigarettes/day) and 15% were diabetic. Ten patients were treated with Statins and 62 with anti-platelet/anticoagulant drugs. ADMA, Norepinephrine, IL-6 and homocysteine were above the upper limit of the corresponding

normal range in 99 (35%), 135 (48%), 196 (69%) and 241 (85%) ESRD patients, respectively. LVMI was on average 64±20 g/m$^{2.7}$ and the large majority of ESRD patients (77%) displayed LVH at echocardiography.

Occurrence of cerebro-vascular events in the study cohort

During the follow-up (range 0.03-131 months), 181 patients died. Forty-seven patients experienced at least one cerebro-vascular event (1 episode in 39 patients, 2 in 6 patients, 3 in 1 patient and 7 in 1 patient). Overall, 61 cerebro-vascular events occurred (5.1 events/100 person-years) and among these 31 were fatal (24 ischemic episodes, 6 hemorrhagic and 1 unclassified) and 30 were non fatal (26 ischemic episodes, 3 hemorrhagic and 1 unclassified). Cerebro-vascular events were ischemic in 50 episodes (82%), hemorrhagic in 9 episodes (15%) and unclassified in the remaining 2 episodes (3%). The proportion of patients with a history of atrial fibrillation was higher in patients with cerebro-vascular events than in those without (9% vs 2%).

Predictors of cerebro-vascular events: univariate analysis

On univariate Cox regression analysis (conditional risk set method), the risk of cerebro-vascular outcomes was directly related to age, smoking, diabetes, BMI, systolic and pulse pressures, triglycerides, haemoglobin, history of stroke/TIA, history of arrhythmia and LVMI (**Table 1**) while norepinephrine, total homocysteine, IL-6 and ADMA failed to predict these events. Treatments with anti-platelet/anticoagulant drugs (P=0.21) and Statins (P=0.74) were unrelated to cerebro-vascular events. Univariate data analysis according to competing risks method produced similar results (data not shown).

Predictors of cerebro-vascular events: multivariate analyses

In a multiple Cox regression model (conditional risk set method) including all univariate predictors of cerebro-vascular events (see Table 1), only smoking, age, haemoglobin, triglycerides, pulse pressure and LVMI maintained a direct and independent relationship with these outcomes (Table 2). These relationships, but that of triglycerides, were confirmed in a competitive risks analysis (Table 3) accounting for non stroke death. Arrhythmia, history of stroke/TIA, systolic arterial pressure, BMI and diabetes were no longer significant predictors of cerebro-vascular outcomes after multivariate data adjustment in both conditional and competitive risk models (Table 2-3). The relationship between haemoglobin and stroke risk did not change also forcing erythropoietin use into the models. Of note, the direct link between haemoglobin and cerebro-vascular events was significantly stronger (P<0.05) than that of the same variable and all-cause death (Table 3, last column).

Table 2 Multivariate Cox regression model for cerebro-vascular events (conditional risk set approach)(19)

	Units of increase	HR and 95% CI	P
Smoking	**0=no;1=yes**	**2.45(1.29-4.65)**	**0.006**
Age	**1 year**	**1.05(1.01-1.08)**	**0.009**
Haemoglobin	**1 g/dL**	**1.28(1.06-1.54)**	**0.01**
Triglycerides	**10 mg/dL**	**1.04(1.01-1.08)**	**0.03**
Pulse pressure	**10 mmHg**	**1.53(1.01-2.33)**	**0.04**
LVMI	**1 g/m$^{2.7}$**	**1.02(1.01-1.04)**	**0.04**
History of arrhythmia	0=no;1=yes	2.42(0.82-7.10)	0.11
History of stroke/TIA	0=no;1=yes	1.88(0.824.29)	0.14
Systolic arterial pressure	10 mmHg	0.80(0.57-1.14)	0.21
Body mass index	1 kg/m^2	1.01(0.93-1.10)	0.73
Diabetes	0=no;1=yes	0.96(0.40-2.31)	0.92

Data are expressed as HR, 95% CI and P value.

Table 3 Multivariate Cox regression model for cerebro-vascular events and death (competing risks approach)(20).

	Units of increase	Cerebro-vascular events	Death	*P value for equality of hazard ratios
Smoking	**0=no;1=yes**	**2.31(1.11-4.81),P=0.025**	**1.61(1.05-2.46),P=0.028**	0.43
Age	**1 year**	**1.05(1.01-1.09),P=0.007**	**1.03(1.01-1.05),P=0.001**	0.36
Pulse pressure	**10 mmHg**	**1.64(1.05-2.56),P=0.03**	**1.08(0.83-1.41),P=0.57**	0.15
Haemoglobin	**1 g/dL**	**1.27(1.02-1.58),P=0.03**	**1.00(0.91-1.10),P=0.98**	**<0.05**
LVMI	**1 g/m$^{2.7}$**	**1.02(1.01-1.04),P=0.037**	**1.03(1.02-1.04),P<0.001**	0.49
Systolic pressure	10 mmHg	0.71(0.35-1.44),P=0.34	0.72(0.37-1.40),P=0.33	0.57
Diabetes	0=no;1=yes	1.44(0.60-3.48),P=0.42	1.57(0.92-2.71),P=0.10	0.88
History of stroke/TIA	0=no;1=yes	1.51(0.64-3.56),P=0.34	1.39(0.85-2.29),P=0.19	0.87
History of arrhythmia	0=no;1=yes	1.60(0.50-5.11),P=0.43	1.39(0.69-2.80),P=0.36	0.84
Triglycerides	10 mg/dL	1.03(0.98-1.08),P=0.25	1.01(0.99-1.04),P=0.30	0.60
Body mass index	1 kg/m^2	1.00(0.92-1.09),P=0.96	0.96(0.91-1.00),P=0.06	0.35

Data are expressed as HR, 95% CI and P value. *P value testing HRs equality of each covariate with two study outcomes.

DISCUSSION

This study for the first time shows that traditional risk factors such as age, smoking and pulse pressure as well as non traditional risk factors like haemoglobin and LV mass by echo-cardiography are independent predictors of cerebro-vascular events in ESRD patients while biomarkers of inflammation, sympathetic activity and endothelial dysfunction are unrelated that these outcomes in this population.

Cerebrovascular outcomes in ESRD patients

According to previous studies in the dialysis population (1,6, 25-26) we found that 17% of total deaths were due to cerebro-vascular events and that the incidence rate of these outcomes was 5.1 events/100 person-years. We also found that 82% of cerebro-vascular events were

ischemic in nature which is again in keeping with previous findings (1,6). The fatality rate of cerebro-vascular events was recently investigated in the CHOICE study (6) which reported a 35% case-fatality in a mixed population of black and white ESRD patients. Our study, the first investigating the fatality rate of cerebro-vascular events in a large cohort composed exclusively of Caucasian ESRD patients, shows that these events were fatal in about one half of cases.

Risk factors for cerebro-vascular outcomes in ESRD patients

Even though in previous studies (9-11,13, 24) we documented that IL-6, homocysteine, ADMA and norepinephrine signal a high cardiovascular risk in the dialysis population, the present analysis shows that they don't predict cerebrovascular events. According to studies in the general population (27) we found that smoking entailed a 2.5 times excess risk for cerebrovascular events also in ESRD patients. Such an observation contrasts with previous studies reporting no relationship between smoking and cerebro-vascular events in ESRD (4-6). However, the follow-up was shorter than that in our study (5 years versus 10 years) in two previous papers (4-5) while in the third study (6) the multi-racial composition of the cohort may have altered the relationship between smoking and cerebrovascular events (28).

Of note, we found that pulse pressure is a significant predictor of cerebrovascular outcomes in ESRD patients which is consistent with previous data (29). To our knowledge, the relationship between haemoglobin and stroke risk in ESRD patients was specifically investigated only by Seliger SL et al. (5) who reported a link between anemia (Hb< 9 g/dL) and stroke risk. In contrast we found that 1 g/dL increase in haemoglobin signals a 27% increase in the risk of cerebro-vascular events, an association that was independent also of erythropoietin use. Our finding is consistent with long term prospective studies in the general population (30-31) where high rather than low haemoglobin is a risk factor for cerebro-vascular events. Studies in the general population (32-34) demonstrated that LV mass is an independent risk factor for cerebro-vascular events but in the CHOICE study in ESRD patients (6) the occurrence of these events was unrelated to LVH. Our study for the first time shows that the predictive power of LVH for cardiovascular outcomes extends to ESRD. We studied an ethnically homogenous cohort of Caucasian ESRD patients and defined LVH on the basis of echocardiograohy while the CHOICE study (6) included a mixed population of black and white ESRD patients and defined LVH on the basis of EKG criteria, a method with a sensitivity much inferior to that of echocardiography.

Our study has limitations. First, the observational nature of our findings precludes definitive conclusions about the nature (causal/non causal) of the associations we registered. Second, even though our study was adequately powered to test independent predictors of cerebro-vascular events, our cohort was relatively small. Therefore, our data await confirmation in larger studies. Strengths of our study are: 1) it is the only one considering IL-6, ADMA, norepinephrine and homocysteine as potential predictors of cerebro-vascular events in ESRD

patients; 2) the accurate patient monitoring that allowed a complete case ascertainment in 96% of patients displaying cerebro-vascular events; 3) the long follow-up period (10 years) and 4) the robustness of statistical methods applied to test the study hypotheses accounting for both failure order (conditional risk set method) and failure types (competing risks analysis).

Perspectives

This study generates the hypothesis that multiple interventions aimed at reducing arterial stiffness, LVMI and smoking as well as at maintaining haemoglobin within the recommended range may have beneficial effects for the prevention of cerebrovascular events in ESRD patients, a hypothesis that needs to be confirmed in specifically designed clinical trials.

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Chapter 4.4

Inflammation and asymmetric dimethyl-arginine (ADMA) for predicting death and cardiovascular events in ESRD patients.

ABSTRACT

Endothelial dysfunction as assessed by asymmetric dimethyl-arginine (ADMA) and inflammation have been consistently linked to atherosclerosis, death and cardiovascular (CV) events in ESRD patients. Inflammation amplifies the effect of ADMA on the severity of atherosclerosis in ESRD patients but it is still unknown whether inflammation and ADMA interact in the high risk of death and CV events in this population.

In a cohort of 225 haemodialysis patients we investigated the interaction between inflammatory biomarkers (CRP and IL-6) and ADMA levels as predictors of death and CV events over an extended follow-up (13 years).

During the follow-up, 160 patients died and 123 had fatal and non fatal CV events. Both on crude and multiple Cox regression analyses, a biological interaction was found between inflammation biomarkers and ADMA for predicting death and fatal and non fatal CV events in ESRD patients. Indeed, the adjusted hazard ratios (HR) for death (HR: 2.18, 95% CI: 1.34-3.54) and CV outcomes (HR: 2.59, 95% CI: 1.47-4.55) of patients with increased CRP and ADMA were higher than those expected in the absence of interaction under the additive model (HR:1.15 and HR:1.97, respectively) and this was also true when the same analysis was carried out by stratifying patients according to IL-6.

These data support the hypothesis that inflammation amplifies the risk of death and CV events associated with high ADMA levels in ESRD patients. These analyses further emphasize the need of intervention studies aimed at attenuating inflammation and high ADMA levels in this population.

Key words: asymmetric dimethylarginine, dialysis, inflammation, interaction.

INTRODUCTION

Over the last decade substantial evidence has been accrued that asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, is consistently related to cardiovascular complications and adverse clinical outcomes in disparate populations (1-14). Indeed, high ADMA was associated with a high risk of death in end stage renal disease (ESRD) patients (2;5) as well as in patients with chronic kidney disease (8-9), peripheral artery disease (10), coronary artery disease (11) and in the Framingham offspring study (14). While it remains uncertain whether the association between ADMA and clinical outcomes is causal in nature (15), a variety of experimental and clinical studies implicate high ADMA in cardiovascular remodelling and dysfunction in ESRD patients (16-17). ADMA infusion impairs vascular reactivity in healthy subjects (18) and high levels of this methyl-arginine are associated with endothelial dysfunction in untreated, uncomplicated, essential hypertensives (19). High ADMA was associated with enlarged atrial volume in the Framingham heart study (20) and with concentric left ventricular hypertrophy in ESRD patients (16). By the same token, ADMA emerged as a robust correlate of intima-media thickness in both general population (21) and in ESRD patients (17).

Several lines of evidence indicate that endothelial dysfunction is intimately associated with chronic inflammation (22-23). In a prospective cohort study in patients with type 2 diabetes an interaction emerged between inflammation (as assessed by circulating levels of C-Reactive Protein) and circulating ADMA for predicting cardiovascular events (24). The possibility that inflammatory cytokines and ADMA may interact in predicting death and adverse CV outcomes in ESRD patients has never been investigated. The issue is of importance because in a previous longitudinal study in the dialysis population we found a strong interaction between CRP and ADMA to predict the worsening in carotid atherosclerosis in ESRD patients (17).

With this background in mind, we investigated the interaction between inflammatory biomarkers (CRP and IL-6) and ADMA levels for predicting long term mortality and cardiovascular events in ESRD patients within the Cardiovascular Risk Extended Evaluation in Dialysis patients (CREED) study cohort.

METHODS

Protocol

The protocol was in conformity to the ethical guidelines of our Institutions and informed consent was obtained from each participant. All blood samples for laboratory tests were taken during a mid-week non-dialysis day, between 8 A.M. and 1 P.M.

Study Cohort

We studied an incident-prevalent cohort of 225 hemodialysis patients (123 M and 102 F) who had been on regular dialysis treatment (RDT) for at least 6 months (median duration of RDT; 43 months; inter-quartile range: 21-109 months). The enrolment criteria in this cohort were no history of congestive heart failure [defined as dyspnea in addition to two of the following conditions - raised jugular pressure, bibasilar crackles, pulmonary venous hypertension or interstitial edema on chest X ray, requiring hospitalization or extra ultra-ultrafiltration], left ventricular ejection fraction >35% and no inter-current or terminal illnesses. Haemodialysis patients were treated thrice weekly with standard bicarbonate dialysis (Na 138 mMol/L, HCO3 35 mMol/L, K 1.5 mMol/L, Ca 1.25 mMol/L, Mg 0.75 mMol/L) either with cuprophan or semi-synthetic membranes (dialysis filters surface area: 1.1-1.7 m2). The average urea Kt/V in these patients was 1.21±0.26. Mortality and cardiovascular outcomes as related to ADMA over a shorter follow-up (5 years) in this cohort where published elsewhere (2).

Follow up

After the initial assessment patients were followed up for 13 years. During the follow-up cardiovascular events (ECG documented anginal episodes and myocardial infarction, heart failure, ECG documented arrhythmia, transient ischemic attacks, stroke and other thrombotic events) and death were accurately recorded. Each death was reviewed and assigned an underlying cause by a panel of 5 physicians. As a part of the review process, all available medical information about each death were collected. This information always included study and hospitalisation records. In the case of an out-of-hospital death family members were interviewed by telephone to better ascertain the circumstances surrounding death.

Laboratory measurements

Blood sampling was performed after an overnight fast between 8.00 a.m. and 10.00 a.m. always during a mid-week non-dialysis day for haemodialysis patients. After 20-30 min of quiet resting in semi-recumbent position samples were taken into chilled EDTA vacutainers, placed immediately on ice, centrifuged within 30 min at -4°C and the plasma stored at -80°C before assay. Serum lipids, albumin, calcium, phosphate, and haemoglobin measurements were made using standard methods in the routine clinical laboratory. The plasma concentrations of ADMA, CRP and homocysteine were measured according to standard methods described elsewhere (2). It should be noted that the ADMA data were obtained in 2000 by HPLC (2). At that time there were still no human plasma reference samples for absolute quantification available, which explains the fact that in most historic studies ADMA-values exceed the values obtained by today's gold standard methods by a factor of two to three. Therefore, when interpreting the data, relative rather than absolute values should be considered. Using a very similar cohort we were recently able to replicate the principal findings obtained with the CREED cohort (5). The outcome results obtained by the new and by the historic HPLC

method were very similar. This indicates that despite the difference in absolute values the correlations and clinical implications based on relative differences in historic (high absolute) or recent (low absolute) ADMA values remain the same. Accordingly we based our present calculations on relative ADMA data (dichotomized at the median). Serum levels of IL-6 were measured de novo by ELISA with the use of Quantikine High Sensitivity kits (R&D Systems Inc, Minneapolis, USA).

Statistical analysis

Data are expressed as mean ± standard deviation, median and inter-quartile range (IQR) or as percent frequency, as appropriate. The relationship between continuous variables was investigated by Pearson product moment correlation coefficient (r) and P value. Variables having a positively skewed distribution were log transformed (lg_{10}) before the correlation study.

The independent predictive value of ADMA, CRP and IL-6 for death and cardiovascular events was analysed by multiple Cox regression analysis. The effect modification of inflammation biomarkers (CRP and IL-6) on the relationship between ADMA and outcomes was investigated by dividing the study population into four categories according to the median values of ADMA and CRP (or IL-6). In relative terms, the effect modification of inflammation biomarkers on the predictive value of ADMA was investigated by multiple Cox regression analyses. In multivariate models we included a categorical variable resulting from the combination of CRP/IL-6 and ADMA (as re-codified according to corresponding median values) as well as Framingham risk factors (age, sex, smoking, diabetes, cholesterol and systolic pressure), background CV complications, anti-hypertensive treatment, factors peculiar to ESRD (dialysis vintage, albumin, haemoglobin and calcium*phosphate) and homocysteine. Biological interaction (synergism) between CRP (or IL-6) and ADMA was defined as a deviation from additivity (25) occurring when the observed hazard ratio (HR) for study outcomes of patients with both high CRP (or high IL-6) and high ADMA was higher than that expected by summing up the hazard ratio of those with high CRP (or high IL-6) and low ADMA or low CRP (or low IL-6) and high ADMA minus one. The excess risk from both exposures in the presence of interaction relative to the excess risk from both exposures in the absence of interaction was assessed by calculating the synergy index (25). The proportionality assumption of Cox models was tested by the analysis of Schoenfeld residuals and no violation was found. The homogeneity of HRs over time associated to key variables (ADMA, CRP and IL-6 and their interaction) was investigated by analysing the interaction of these variables and time. Data are expressed as hazard ratio (HR), 95% confidence intervals (CI) and P values. All calculations were done by a standard statistical package (SPSS for Windows Version 9.0.1, Chicago, Illinois, USA).

RESULTS

The main demographic and clinical characteristics of patients included in the study are detailed in Table 1. The prevalence of diabetes mellitus in this cohort was 15% (i.e. 34 patients out of 225). Eighty-three patients were habitual smokers (21±16 cigarettes/day). One hundred and twenty-two patients were on treatment with erythropoietin and 82 patients were being treated with anti-hypertensive drugs (58 on mono-therapy with ACE inhibitors, AT-1 antagonists, Calcium channel blockers, alpha and beta-blockers and 24 on double or triple therapy with various combinations of these drugs).

ADMA, C RP and IL-6 levels in the study population

Circulating levels of ADMA (median: 2.4 µMol/L, inter-quartile range: 1.6-3.8 µMol/L), CRP (7.4 mg/L, 3.4-16.4 mg/L) and IL-6 (5.0 pg/mL, 2.7-9.2 pg/mL) were above the upper limit of the corresponding normal ranges in 133 (59%), 124 (55%) and 32 (14%) ESRD patients,

Table 1 Main demographic, somatometric and clinical characteristics of the study population.

	Whole cohort (n=225)	CRP<7.4 mg/L and ADMA<2.4 µMol/L (n=64)	CRP≥7.4 mg/L and ADMA<2.4 µMol/L (n=47)	CRP<7.4 mg/L and ADMA≥2.4 µMol/L (n=50)	CRP≥7.4 mg/L and ADMA≥2.4 µMol/L (n=64)	**P (ANOVA)**
Age (years)	60±15	59±17	65±11	56±14	59±15	0.02
Dialysis vintage (months)	42(21-109)	27(14-61)	43(25-77)	86(23-172)	46(24-129)	<0.001
Male sex n. (%)	123(55%)	34(53%)	21(45%)	24(51%)	43(67%)	0.10
Smokers n. (%)	83(37%)	21(33%)	15(32%)	19(40%)	28(44%)	0.48
Diabetics n. (%)	34(15%)	9(14%)	11(23%)	3(6%)	11(17%)	0.14
With CV comorbidities n. (%)	113(50%)	24(38%)	31(66%)	17(36%)	40(63%)	<0.001
On anti-hypertensive treatment n. (%)	82(36%)	20(31%)	12(26%)	19(40%)	30(47%)	0.09
Systolic pressure (mmHg)	139±25	137±25	142±30	139±26	141±21	0.62
Diastolic pressure (mmHg)	76±13	77±13	76±13	75±12	76±13	0.94
Cholesterol (mg/dL)	208±58	192±44	217±72	218±60	209±55	0.06
Haemoglobin (g/L)	106±19	110±19	106±15	109±19	105±20	0.32
Calcium *Phosphate ($mMol^2/L^2$)	4.52±1.12	4.59±1.19	4.48±1.11	4.41±1.04	4.57±1.14	0.82
Albumin (g/L)	42±5	43±4	41±4	42±5	41±6	0.14
CRP (mg/L)	7.5(3.4-16.4)	3.4(3.4-3.5)	20.6(13.7-30.7)	3.4(3.4-4.3)	15.7(11.5-27.7)	<0.001
IL-6 (pg/mL)	5.0(2.7-9.2)	3.3(2.6-5.3)	4.8(2.9-10.2)	4.4(2.2-8.2)	7.7(4.9-11.8)	<0.001
ADMA (µMol/L)	2.52(1.58-3.85)	1.63(1.06-1.98)	1.56(1.10-1.97)	3.55(2.89-4.69)	3.99(3.33-5.24)	<0.001
Homocysteine (µMol/L)	27.0(19.4-42.7)	29.3(20.3-52.0)	25.4(20.0-38.5)	26.2(18.2-44.7)	27.3(19.4-42.7)	0.41

Data are expressed as mean± SD, median and inter-quartile range or as percent frequency, as appropriate. Patients were grouped according to the median values of CRP and ADMA.

respectively. On univariate analyses, plasma levels of ADMA were significantly related to those of CRP (r=0.13, P=0.046) and IL 6 (r=0.18, P=0.009). A stratified analysis according to the median values of CRP (below/above: 7.4 mg/dL) and ADMA (below/above: 2.4 µMol/**L) (Table 1) showed** that age (P=0.02), dialysis vintage (P<0.001) and the proportion of patients with background CV complications (P<0.001) significantly differed among groups while cholesterol (P=0.06) and the proportion of patients treated with anti-hypertensive drugs (P=0.09) just failed to reach the statistical significance. Similar results were obtained by stratifying patients according to ADMA and IL-6 (below/above 5 pg/mL) (data not shown).

Effect modification of inflammation on the ADMA-outcomes relationship(Fig. 1-Fig. 3)

During the follow-up period (156 months), 160 patients died (15 deaths/100 person-years) and 123 had fatal and non fatal CV events (13 events/100 person-years). In Cox models including ADMA and CRP (or IL-6) as well as a series of potential confounders (Table 2), these biomarkers significantly predicted death (ADMA: P<0.001; CRP: P=0.008; IL-6: P=0.002) while ADMA (P=0.001) was the sole biomarker predicting fatal and non fatal CV events (Table 2). The relationship between ADMA and the incidence rate of mortality and CV outcomes was closely dependent on CRP categories (effect modification of ADMA by CRP) (Fig. 1), the incidence rate of mortality and CV events being maximal in patients with high ADMA (≥ 2.4 µMol/L) and high CRP (≥7.4 mg/dL) and minimal in patients with low ADMA (<2.4 µMol/L) and low CRP (<7.4 mg/dL) and this was also true when the same analysis was carried out

Table 2 Cox regression analyses of the main effect of ADMA, CRP and IL-6 on the incidence rate of mortality (**a**) and fatal and non fatal CV events (**b**).

a) All-cause mortality

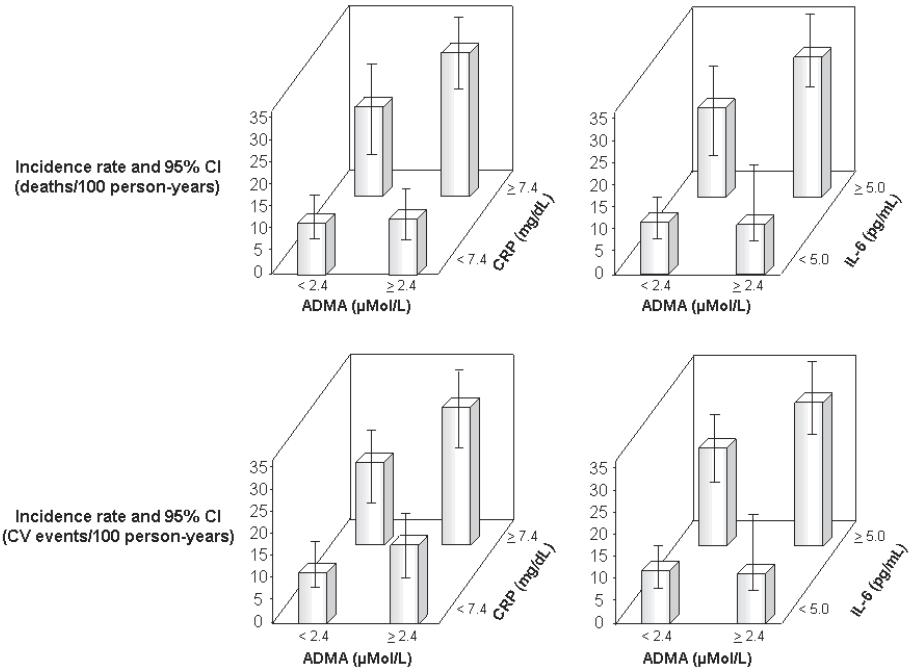
Variables (Units of increase)	ADMA and CRP based model *(Hazard ratio, 95% CI, and P)*	ADMA and IL-6 based model *(Hazard ratio, 95% CI, and P)*
Age (1 year)	1.04(1.02-1.06), P<0.001	1.04(1.02-1.05), P<0.001
Male sex	1.67(1.07-2.61), P=0.02	1.55(0.99-2.41), P=0.06
Smoking	1.22(0.80-1.86), P=0.36	1.24(0.81-1.89), P=0.32
Diabetes	2.26(1.45-3.51), P<0.001	2.42(1.55-3.78), P<0.001
Cholesterol (20 mg/dL)	1.01(0.94-1.08), P=0.81	1.02(0.95-1.09), P=0.64
Systolic pressure (1 mmHg)	1.00(0.99-1.01), P=0.006	1.00(0.99-1.01), P =0.94
CV comorbidities (0=no; 1=yes)	1.65(1.16-2.36), P=0.006	1.57(1.10-2.23), P=0.01
Anti-hypertensive treatment (0=no; 1=yes)	0.89(0.60-1.31), P=0.56	0.87(0.59-1.29), P=0.50
Dialysis vintage (10 months)	1.00(0.98-1.03), P=0.98	1.00(0.97-1.02), P=0.87
Albumin (1 g/L)	0.97(0.93-1.01), P=0.14	0.98(0.94-1.02), P=0.27
Haemoglobin (1 g/L)	1.01(0.99-1.02), P=0.08	1.01(0.99-1.02), P=0.11
Calcium *Phosphate (1 $mMol^2/L^2$)	1.04(0.89-1.21), P=0.66	1.06(0.91-1.24), P=0.47
Homocysteine (10 µMol/L)	1.04(0.98-1.10), P=0.21	1.03(0.98-1.09), P=0.22
ADMA (1 µMol/L)	1.22(1.12-1.34), P<0.001	1.21(1.10-1.32), P<0.001
CRP (5 mg/L)	1.04(1.01-1.07), P=0.008	…
IL-6 (1 pg/mL)	…	1.03(1.01-1.05), P=0.002

b) Fatal and non fatal CV events

Variables (Units of increase)	ADMA and CRP based model *(Hazard ratio, 95% CI, and P)*	ADMA and IL-6 based model *(Hazard ratio, 95% CI, and P)*
Age (1 year)	1.03(1.02-1.06), P<0.001	1.03(1.02-1.06), P<0.001
Male sex	1.36(0.82-2.27), P=0.23	1.34(0.80-2.24), P=0.27
Smoking	1.59(0.99-2.56), P=0.06	1.61(1.00-2.58), P=0.05
Diabetes	1.92(1.16-3.18), P=0.01	1.94(1.17-3.23), P=0.01
Cholesterol (20 mg/dL)	1.04(0.96-1.12), P=0.32	1.04(0.96-1.12), P=0.31
Systolic pressure (1 mmHg)	1.01(0.99-1.02), P=0.86	1.00(0.99-2.58), P=0.87
CV comorbidities (0=no; 1=yes)	1.56(1.03-2.35), P=0.04	1.55(1.02-2.34), P=0.04
Anti-hypertensive treatment (0=no; 1=yes)	2.02(1.31-3.12), P=0.002	2.00(1.29-3.09), P=0.002
Dialysis vintage (10 months)	1.03(0.991.06), P=0.06	1.03(0.99-1.06), P=0.06
Albumin (1 g/L)	0.98(0.94-1.03), P=0.48	0.98(0.94-1.03), P=0.52
Haemoglobin (1 g/L)	1.01(0.99-1.02), P=0.28	1.01(0.99-1.02), P=0.30
Calcium *Phosphate (1 $mMol^2/L^2$)	1.05(0.88-1.25), P=0.60	1.05(0.89-1.25), P=0.56
Homocysteine (10 µMol/L)	1.02(0.96-1.09), P=0.51	1.02(0.96-1.09), P=0.52
ADMA (1 µMol/L)	1.18(1.07-1.30), P=0.001	1.17(1.06-1.29), P=0.001
CRP (1 mg/L)	1.01(0.97-1.06), P=0.61	…
IL-6 (1 pg/mL)	…	1.01(0.98-1.03), P=0.60

Data are hazard ratio, 95% confidence intervals and P value.

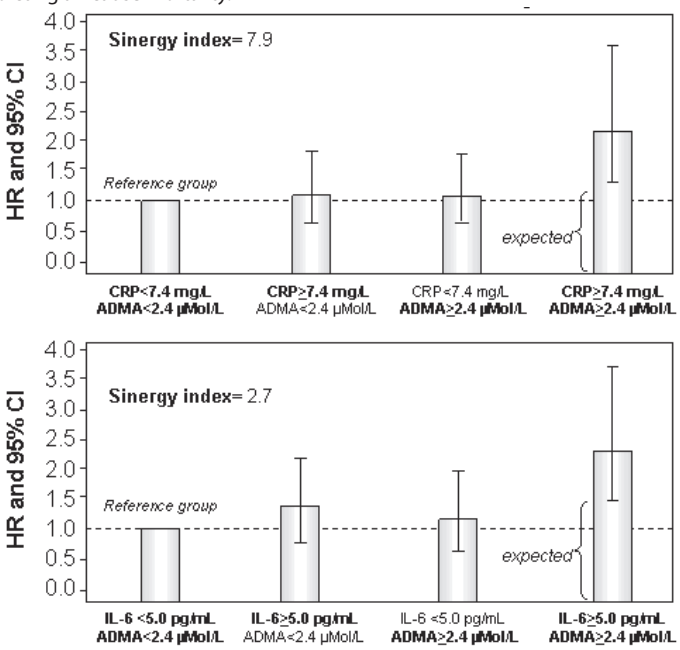
Figure 1. Relationship of ADMA, CRP and IL-6 (as recodified according to the corresponding median value) and study outcomes (all-cause mortality and fatal and non fatal CV events).



Data are crude (unadjusted) incidence rates and 95% confidence intervals. Patients are divided into four groups according to the median value of ADMA and CRP (or IL-6).

according to IL-6 (Fig. 1). Of note, in patients with low levels of CRP or IL-6 no excess risk for death and CV events was associated to high levels of ADMA (Fig. 1). However, after data adjustment for potential confounders, patients with high circulating levels of ADMA and low CRP (or IL-6) tended to have an increased risk of fatal and non fatal CV events (Fig. 3) Accordingly, in multiple Cox regression models (Fig. 2-3) a biological interaction was found between inflammation biomarkers and ADMA for predicting all-cause mortality and fatal and non fatal CV events. Indeed, the adjusted hazard ratios for death (Fig. 2) and CV outcomes (Fig. 3) of patients with increased ADMA and CRP were higher than those expected in the absence of interaction under the additive model and this was also true when the same analysis was carried out by stratifying according to IL-6 (Fig. 2-3). Remarkably, the excess risk for death and CV events due to the interaction (synergy index) was from 1.6 to 7.9 times higher than

Figure 2. Biological interaction between CRP, IL-6 and ADMA (below/above the corresponding median values) for predicting all-cause mortality.



Data are expressed as hazard ratio and 95% confidence intervals. Data were adjusted for age, sex, smoking, diabetes, cholesterol, systolic pressure, background CV complications, anti-hypertensive treatment, dialysis vintage, albumin, haemoglobin, calcium*phosphate and homocysteine. Deviation from additivity or presence of biological interaction (or synergism)(25) was assessed by comparing the observed joint effect of high CRP (or IL-6) and high ADMA levels with that expected in the absence of interaction (see below).

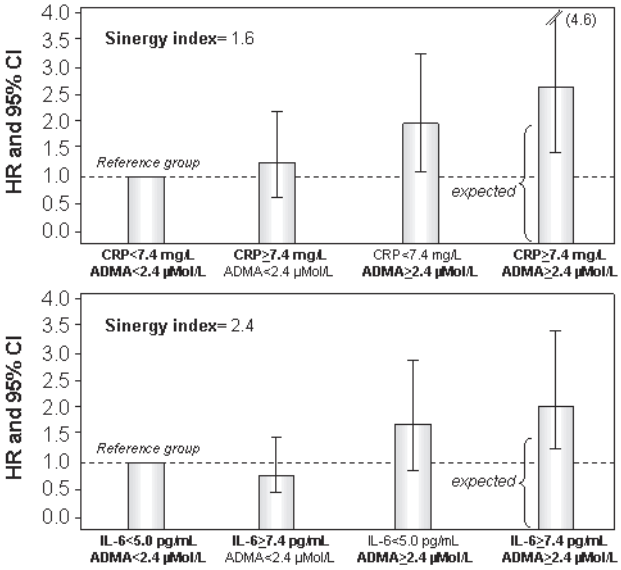
- Expected effect of CRP and ADMA in the absence of interaction:

$(HR_{ADMA < 2.4\ \mu Mol/L \text{ and } CRP \geq 7,4\ mg/L} + HR_{ADMA \geq 2.4\ \mu Mol/L \text{ and } CRP < 7,4\ mg/L} - 1) = 1.08 + 1.07 - 1 = 1.15$

- Expected effect of IL-6 and ADMA in the absence of interaction:

$(HR_{ADMA < 2.4\ \mu Mol/L \text{ and IL-6} \geq 5,0\ mg/L} + HR_{ADMA \geq 2.4\ \mu Mol/L \text{ and IL-6} < 5.0\ mg/L} - 1) = 1.36 + 1.12 - 1 = 1.48$

Figure 3. Biological interaction between CRP, IL-6 and ADMA (below/above the corresponding median values) for predicting fatal and non fatal CV events.



Data are expressed as hazard ratio and 95% confidence intervals. Data were adjusted for age, sex, smoking, diabetes, cholesterol, systolic pressure, background CV complications, anti-hypertensive treatment, dialysis vintage, albumin, haemoglobin, calcium*phosphate and homocysteine. Deviation from additivity or presence of biological interaction (or synergism) (25) was assessed by comparing the observed joint effect of high CRP (or IL-6) and high ADMA levels with that expected in the absence of interaction (see below).

- Expected effect of CRP and ADMA in the absence of interaction:

$(HR_{ADMA < 2.4\ \mu Mol/L \text{ and } CRP \geq 7,4\ mg/L} + HR_{ADMA \geq 2.4\ \mu Mol/L \text{ and } CRP < 7,4\ mg/L} - 1) = 1.13 + 1.84 - 1 = 1.97$

- Expected effect of IL-6 and ADMA in the absence of interaction:

$(HR_{ADMA < 2.4\ \mu Mol/L \text{ and IL-6} \geq 5,0\ mg/L} + HR_{ADMA \geq 2.4\ \mu Mol/L \text{ and IL-6} < 5.0\ mg/L} - 1) = 0.78 + 1.64 - 1 = 1.42$

that portended by high ADMA and high inflammation markers in the absence of interaction (Fig. 2-3).

The risk excess for death and fatal and non fatal CV events associated to high circulating levels of ADMA and inflammatory biomarkers (CRP and IL-6) did not change throughout the follow-up period (P=NS), implying that the effect modification of CRP and IL-6 on the relationship between ADMA and study outcomes remained stable and statistically significant over time.

DISCUSSION

This study shows that, independently of traditional and non traditional CV risk factors, inflammation amplifies the risk of death and CV events associated with high plasma ADMA in

dialysis patients and that in patients with relatively low CRP and IL-6 no excess risk for death and CV outcomes is associated to high levels of ADMA.

Inflammation and endothelial dysfunction in ESRD patients

Inflammation in ESRD patients is a multi-factorial problem (22). Both dialysis related and dialysis independent factors may promote inflammation by stimulating the synthesis and/or release of several pro-inflammatory cytokines like CRP, IL-1, IL-6, TNF-α and interferon-γ (IFN- γ) (23). Impaired endothelium-dependent vasodilation is a hallmark in patients with ESRD (26) and high ADMA levels are considered as a major factor in endothelial dysfunction in chronic renal failure (26-27). In our study, the majority of ESRD patients had circulating levels of ADMA and CRP above the corresponding upper limit of the normal range (59% and 55%, respectively). Circulating levels of ADMA were directly and significantly related to those of CRP and IL-6 indicating that inflammation and endothelial dysfunction are parallel processes in ESRD patients. These relationships may reflect a causal link because in patients with familial Mediterranean fever both ADMA and CRP levels show concomitant increase during the acute phase of the disease to revert to normal after the resolution of fever (28).

Interaction between ADMA and inflammation

The relationship between high ADMA and CRP is context dependent. Indeed, in acute sepsis ADMA is down-regulated (29) while high ADMA levels are consistently associated with biomarkers of inflammation in a variety of chronic conditions including untreated essential hypertension (30), glucose intolerance (31), familial Mediterranean fever (28) and inflammatory bowel diseases (32). In vitro, ADMA induces TNF-alpha production via ROS/NF-kappaB dependent pathway (33). On the other hand the generation of reactive oxygen species, an important initial event in inflammation, inhibits the enzyme that degrades ADMA (Dimethylarginine dimethylaminohydrolase, DDAH) facilitating local and/or systemic ADMA accumulation. ADMA in turn increases the generation of the downstream pro-inflammatory mediators TNF-alpha and IL-8, and activates the NF-kB pathway and the binding of monocytes to endothelial cells (34). Even though a steady state functional link between ADMA and inflammation has already been described in ESRD patients (2), the prognostic value of the interaction between these two factors for predicting death and cardiovascular outcomes in these patients has never been investigated. In the present study, patients with both high ADMA and CRP (or high IL-6) levels had higher risk of death and fatal and non fatal cardiovascular events than those with only one elevated biomarker and such an excess risk exceeded that one would expect by adding the individual risks of these factors (synergism). This finding is in keeping with the results of a longitudinal study by our group (17) in which we found a synergic interaction between CRP and ADMA to explain the progression of carotid atherosclerosis in ESRD patients (17). In the AURORA study (35) treatment with Rosuvastatin reduced C-Reactive protein but this drug had no effect on the incidence rate of death and

cardiovascular events in ESRD patients. Rosuvastatin is one of the most potent ADMA modifiers and improvement in endothelial dysfunction in patients with hypercholesterolemia goes along with the ADMA-lowering effect of this drug (36). Secondary analyses in AURORA in patients with high ADMA and CRP may allow preliminary testing the hypothesis generated by the present study and similar analysis may be performed in the 4D study (37) and in the ongoing SHARP trial (38). The main limitation of our study is its observational nature that precludes the possibility to draw definitive conclusions about the nature (causal/non causal) of the relationships we found. Strengths of our hypothesis-generating study are the adequate sample size, the robustness of the statistical approach, the internal coherence and biological plausibility of the results.

In conclusion, inflammation and endothelial dysfunction as assessed by circulating levels of ADMA have an independent, synergic effect for explaining all cause mortality and fatal and non fatal cardiovascular events in ESRD patients. These results generate the hypothesis that interventions aimed at attenuating/preventing endothelial dysfunction and inflammation may attenuate the exceedingly high CV risk burden of these patients.

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Chapter 5

Discussion

DISCUSSION

Studies included in this thesis show that left atrial volume and its circulating biomarkers play an important role for rik stratification and risk monitoring in end stage renal disease patients and underlie the importance of traditional and non traditional risk factors in the high risk of cerebro-vascular and cardiovascular events in the dialysis population. Furthermore, this thesis provides an insight on the etiological role of smoking as a potential, unsuspected, cause of hyperparathyroidism as well as of aging in the pathogenetic pathway leading to left ventricular hypertrophy and left ventricular systolic dysfunction in dialysis patients.

Left Atrial Volume and its Biomarkers in Dialysis Patients

Data analyses in the CREED database show that the volume of the left atrium is markedly increased in patients with ESRD as compared to age and sex matched control subjects and independently associated with left ventricular hypertrophy (LVH) and with LV systolic and diastolic dysfunction. Furthermore, height$^{2.7}$- indexed left atrial volume (LAV) adds independent predictive value for mortality over and beyond LV mass and function and other risk factors in ESRD patients like age, gender and blood pressure. Left atrial size is directly related with LV mass (1) and it is therefore possible that the predictive power of left atrial (LA) size just depends on the fact that this measurement reflects LV mass. Indeed, like in the Framingham heart study (2) we found that the predictive power for death of un-indexed and body surface area (BSA)-indexed LA volume became non significant after the inclusion of LV mass into the predictive model. Yet height$^{2.7}$-indexed LA volume retained an independent predictive power for this outcome. We have previously shown that appropriately indexing LV mass is of particular importance in ESRD and on theoretical grounds the same advantage should also apply to atrial volume (3). Malnutrition is very frequent in patients undergoing dialysis, and body weight is substantially reduced at all height groupings in these patients in comparison to normal individuals (4) which may lead to an overestimation of LAV when cardiac measurements are indexed by BSA. On the other hand, expanded extracellular volume (which increases body weight) may produce an underestimate of LAV because augments the denominator (i.e. BSA). Thus, the estimation of LAV may be distorted in an unpredictable way and in opposite directions by malnutrition and extracellular volume expansion when the BSA index is used in dialysis patients. The present data extend to LAV the importance of the indexation by height proposed by the De Simone for LV mass (3,5), at least in ESRD patients. Another novel finding of our studies is that progressive increase in left atrial volume predicts incident CV events in dialysis patients independently of age and of the corresponding baseline measurement of left atrial volume and of left ventricular mass and function. This observation indicates that monitoring LA size by echocardiography provides independent prognostic information in ESRD patients thus underscoring the relevance of

repeated measurements of this echocardiographic measurement in the clinical management of these patients.

To surrogate information by echocardiography, several studies investigated the diagnostic and prognostic value for major clinical events of biomarkers of left ventricular mass and function (6). We found that brain natriuretic peptide (BNP) displayed a stronger association with LAV changes over time than atrial natriuretic peptide (ANP). In particular, the additional predictive value for progressive LAV enlargement of BNP above established risk factors (including age) was higher (+12%) than that provided by ANP (+8%). Notably, this additional prognostic information by BNP was also superior to that of echocardiographic parameters of LV mass (+5%) and function (+8%). We also found that the simultaneous measurements of BNP and NE was marginally superior to the ANP-BNP combination and that both combinations were better than isolated measurements of the same compounds. BNP and ANP are strongly interrelated and therefore provide overlapping predictive information. Whether the additional information provided by the multiple biomarkers strategy translates into better clinical outcomes is an important clinical question that remains to be investigated in future clinical trials.

Risk factors for hyperparathyroidism and age-related risk factors for left ventricular hypertrophy and dysfunction in patients with End Stage Renal Diseases.

Hyperparathyroidism is a frequent complication in ESRD and a risk factor for left ventricular hypertrophy and cardiac remodelling in the dialysis population (7). We found that heavy cigarette smoking, independently of age and other specific risk factors, is associated with high circulating levels of serum intact PTH and $PTH_{1\text{-}84}$ in a carefully selected dialysis population of non-diabetic patients, never exposed to aluminium and beta blockers. Overall, our study is "hypothesis-generating" rather than "hypothesis-testing" and observational (follow-up and longitudinal), mechanistic and interventional studies are needed to confirm the link between smoking and high PTH and to assess the nature (causal or non causal) of this link in ESRD patients.

Observations in the CREED database revealed that in ESRD patients the relationship between age and cardiomyopathy is largely dependent on age-related risk factors and suggest that interventions focused on modifiable risk factors linked to age (like malnutrition/inflammation and arterial rigidity) could attenuate the detrimental effect of aging on cardiovascular risk in the dialysis population. Left ventricular (LV) hypertrophy and LV systolic dysfunction are pervasive alterations in ESRD patients and represent important risk factors for death and cardiovascular events in the dialysis population (8-9). In the last decades, a variety of risk factors for LVH and LV dysfunction have been identified in dialysis patients but no study specifically focused on age-related risk factors for these alterations in ESRD patients. In our study, on crude analysis age resulted to be a significant correlate of LV hypertrophy and LV dysfunction in ESRD patients. Albumin, pulse pressure, haemoglobin, heart rate, dia-

betes and cardiovascular comorbidities were associated with both age and LV mass and/or LV systolic function. These parallel associations suggest that these well recognised risk factors for cardiomyopathy in ESRD may be the main drivers of the age-dependent increase in LV mass and dysfunction in the dialysis population. To test this hypothesis we entered these factors in the multiple regression models aimed at investigating the variability in LV mass and function. Remarkably, after the inclusion of these factors into these models, age was no longer a significant predictor of LVMI and mwFS suggesting that the link between aging and alterations in LV mass and function was largely dependent on these age-related risk factors.

Risk Factors of cerebro-vascular events in Patients with End Stage Renal Diseases

Our study for the first time shows that traditional risk factors such as age, smoking and pulse pressure as well as non traditional risk factors like haemoglobin and LV mass by echocardiography are independent predictors of cerebro-vascular events in ESRD patients while biomarkers of inflammation, sympathetic activity and endothelial dysfunction are unrelated that these outcomes in this population. Even though in previous studies (10-11) we documented that IL-6, homocysteine, asymmetric dimethyl-arginine (ADMA) and norepinephrine signal a high cardiovascular risk in the dialysis population, the present analysis shows that they don't predict cerebrovascular events. Our study generates the hypothesis that multiple interventions aimed at reducing arterial stiffness, LVMI and smoking as well as at maintaining haemoglobin within the recommended range may have beneficial effects for the prevention of cerebrovascular events in ESRD patients, a hypothesis that needs to be confirmed in specifically designed clinical trials.

Synergism between inflammation and high levels of asymmetric dimethyl-arginine (ADMA) for predicting death and cardiovascular events in end stage renal diseases (ESRD) patients.

Our findings indicate that, independently of age and of traditional and non traditional CV risk factors, inflammation amplifies the risk of death and cardiovascular events associated with high plasma ADMA in dialysis patients and that in patients with relatively low CRP and IL-6 no excess risk for death and CV outcomes is associated to high levels of ADMA. In our study, circulating levels of ADMA were directly and significantly related to those of CRP and IL-6 indicating that inflammation and endothelial dysfunction are parallel processes in ESRD patients (12). These relationships may reflect a causal link because in patients with familial Mediterranean fever both ADMA and CRP levels show concomitant increase during the acute phase of the disease to revert to normal after the resolution of fever (13). The relationship between high ADMA and CRP is context dependent. Indeed, in acute sepsis ADMA is down-regulated (14) while high ADMA levels are consistently associated with biomarkers of inflammation in a variety of chronic conditions. In vitro, ADMA induces TNF-alpha production via ROS/NF-kappaB dependent pathway (15). On the other hand the generation of

reactive oxygen species, an important initial event in inflammation, inhibits the enzyme that degrades ADMA (Dimethylarginine dimethylaminohydrolase, DDAH) facilitating local and/or systemic ADMA accumulation. ADMA in turn increases the generation of the downstream pro-inflammatory mediators TNF-alpha and IL-8, and activates the NF-kB pathway and the binding of monocytes to endothelial cells (16). In our study, patients with both high ADMA and CRP (or high IL-6) levels had higher risk of death and fatal and non fatal cardiovascular events than those with only one elevated biomarker and such an excess risk exceeded that one would expect by adding the individual risks of these factors (synergism). This finding is in keeping with the results of a longitudinal study by our group (17) in which we found a synergic interaction between CRP and ADMA to explain the progression of carotid atherosclerosis in ESRD patients (17). In the AURORA study (18) treatment with Rosuvastatin reduced C-Reactive protein but this drug had no effect on the incidence rate of death and cardiovascular events in ESRD patients. Rosuvastatin is one of the most potent ADMA modifiers and improvement in endothelial dysfunction in patients with hypercholesterolemia goes along with the ADMA-lowering effect of this drug (19). Secondary analyses in AURORA in patients with high ADMA and CRP may allow preliminary testing the hypothesis generated by the present study and similar analysis may be performed in the 4D study (20) and in the ongoing SHARP trial (21). In the aggregate, our study generates the hypothesis that interventions aimed at attenuating/preventing endothelial dysfunction and inflammation may attenuate the exceedingly high CV risk burden of these patients, a possibility that deserves to be specifically investigated in a randomised clinical trial.

Future studies

The main limitation of the CREED study is its observational nature. An important challenge now is to plan specific intervational studies in order to confirm the causal role of all risk factors identified by this project.

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SUMMARY

An extensive data analysis in the CREED database shows that height$^{2.7}$- indexed left atrial volume (LAV) has an independent predictive value for mortality over and beyond left ventricular (LV) mass and function in patients with end stage renal disease (ESRD) and that the progressive increase in LAV over time predicts incident CV events independently of the corresponding baseline measurement of LAV and of LV mass and function in the dialysis population. Furthermore, brain natriuretic peptide (BNP) and atrial natriuretic peptide (ANP) are strongly related to LAV and predict longitudinal changes in LAV in ESRD patients suggesting that the measurement of the plasma concentration of these biomarkers might be useful for risk stratification and for guiding treatment in dialysis patients. We also found that heavy cigarette smoking, independently of age and other specific risk factors, is directly associated with hyperparathyroidism (a well recognized risk factor for cardiac alterations in ESRD patients) while studies performed so far in the general population mainly showed that smoking is inversely rather than directly related to circulating levels of parathormone. Observations in the CREED database also revealed that in ESRD patients the relationship between age and cardiomyopathy is largely dependent on age-related risk factors suggesting that interventions focused on modifiable risk factors linked to age (like malnutrition/inflammation and arterial rigidity) could attenuate the detrimental effect of aging on cardiovascular risk in the dialysis population. We also documented for the first time that traditional risk factors such as age, smoking and pulse pressure as well as non traditional risk factors like haemoglobin and left ventricular mass by echocardiography are independent predictors of cerebro-vascular events in ESRD patients while biomarkers of inflammation, sympathetic activity and endothelial dysfunction are unrelated that these outcomes in this population. These observations generate the hypothesis that multiple interventions aimed at reducing arterial stiffness, LVMI and smoking as well as at maintaining haemoglobin within the recommended range may have beneficial effects for the prevention of cerebrovascular events in ESRD patients, a hypothesis that needs to be confirmed in specifically designed clinical trials. Eventually, we demonstrated that, independently of age and of traditional and non traditional cardiovascular risk factors, inflammation amplifies the risk of death and cardiovascular events associated with high plasma asymmetric dimethyl-arginine (ADMA) in dialysis patients and that in patients with relatively low C-Reactive Protein (CRP) and IL-6 no excess risk for death and cardiovascular outcomes is associated to high levels of ADMA.

SAMENVATTING

Een omvangrijke data analyse van gegevens uit de CREED database laat zien dat hoogte-geindexeerd linker atrium volume (LAV) een onafhankelijke voorspeller is van mortaliteit bij patiënten met eind stadium nierfalen. De voorspellende waarde van LAV bleek groter dan de voorspellende waarde van de linker ventrikel (LV) massa en functie. De toename van LAV over de tijd voorspelt de incidentie van cardiovasculaire accidenten bij dialyse patiënten, onafhankelijk van de uitgangswaarden van LAV en LV massa en functie. Daarnaast bleken het brain natriuretic peptide (BNP) en het atrial natriuretic peptide (ANP) sterk gerelateerd te zijn aan LAV en kunnen zij longitudinale veranderingen in LAV voorspellen. Dit suggereert dat het meten van de plasma concentraties van deze biomarkers zinvol kan zijn bij het stratificeren van risicogroepen en het vormgeven van de behandeling van dialyse patiënten.

Ook vonden we dat zwaar roken direct geassocieerd is met hyperparathyryodisme (een bekende risicofactor voor cardiale veranderingen in patiënten met eind stadium nierfalen), onafhankelijk van leeftijd of andere specifieke risico factoren. Dit terwijl eerdere studies in de algemene bevolking lieten zien dat roken juist omgekeerd gerelateerd is aan de hoeveelheid circulerend parathormoon. Analyse van de CREED database maakte tevens duidelijk dat de relatie tussen leeftijd en cardiomyopathie bij patiënten met eind stadium nierfalen voor een groot deel berust op leeftijd-gerelateerde factoren. Dit suggereert dat interventies op dit gebied (bijvoorbeeld gericht op het voorkomen van ondervoeding, ontsteking en arteriële rigiditeit) de schadelijke effecten van veroudering op het cardiovasculair risico bij patiënten met eindstadium nierfalen zouden kunnen verminderen.

Wij beschreven voor het eerst dat bekende risico factoren zoals leeftijd, roken en polsdruk evenals minder bekende risicofactoren zoals het hemoglobine en de echografisch bepaalde LV massa, onafhankelijke risico voorspellers zijn van cerebro-vasculaire accidenten bij patienten met eindstadium nierfalen. Daarentegen bleken de variabelen ontstekingsparameters, sympatische activiteit en endotheel dysfunctie geen relatie met deze uitkomsten te hebben.

Deze observaties hebben geleid tot de hypothese dat multiple interventies gericht op het verminderen van arteriële rigiditeit, LVMI, roken en het op niveau houden van het hemoglobine-level, gunstige effecten kan hebben op de preventie van cerebrovasculaire accidenten bij patiënten met eind stadium nierfalen. Deze hypothese moet nog bevestigd worden door middel van gerichte klinische studies.

Tenslotte toonden we aan dat bij dialyse patiënten het risico op overlijden en cardiovasculaire accidenten geasscocieerd met hoge plasma waarden van asymmetrisch dimethylarginine (AMDA), vergroot wordt door ontsteking. Deze associatie bestaat onafhankelijk van leeftijd en cardiovasculaire risico-factoren. Bij patiënten met een relatief laag C-reactief proteine (CRP) en IL-6 was geen verhoogd risico aantoonbaar.

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PhD Training

Research Skills

- Biomedical English
- Master of Science in Clinical Epidemiology, Netherlands Institute for Health Sciences, Rotterdam The Netherlands

In-Depth Courses

- Intervention Research and Clinical Trials
- Diagnostic Research
- Prognosis Research
- Advanced Topics in Decision-making in Medicine
- Advances in Population-based Studies of Complex Genetic Disorders
- Genetic Linkage Analysis: Model-free Analysis
- Bayesian Statistics
- Prognostic Research
- Principles of Genetic Epidemiology
- Causal Inference
- Clinical Decision Analysis
- Clinical Trials
- Topics in Health and Diseases in the Elderly

Invited lectures and Seminars

- Problem solving for missing data. XLVIII ERA-EDTA European Congress. Prague (Czech Republic), June 23th, 2011.
- The importance of epidemiology in Nephrology. Venice (Italy), October 14th, 2011.
- Interpretation of study results: focus on biostatistics. International course of biostatistics by the European Renal Association - European Dialysis and Transplantation Association. Leiden (The Netherlands), June 14th, 2012

International conferences

XLVII Congress of European Renal Association - European Dialysis Transplantation Association. Munich, Germany, June 25-27th, 2010.

XLVIII Congress of European Renal Association - European Dialysis Transplantation Association, Prague, Czech Republic, June 22-26 2011.

49th Congress of European Renal Association - European Dialysis Transplantation Association, Paris, France, May 24-27th, 2012.

Other

Reviewer for

- American Journal of Hypertension.
- Nephrology Dialysis and Transplantation. (Editorial Board)
- American Journal of Kidney Disease.
- Journal of Nephrology. (Editorial Board)
- Kidney & Blood Pressure Research.
- Biomed Central-series Journal.
- European Journal of Neurology.
- Nature Reviews Nephrology.
- Nutrition, Metabolism and Cardiovascular Diseases.
- Clinical Journal of the American Society of Nephrology.
- Case Reports in Vascular Medicine.
- Journal of Artificial Organs.
- Aging, Clinical and Experimental Research

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Giovanni Luigi Tripepi was born in Reggio Calabria (Italy) on March 31st 1972. In 2005 he obtained the University Degree of Doctor in Statistical, Demographic and Social Sciences (University of Messina). After that, he started (August 2007) the Master of Science in Clinical Epidemiology at the Netherlands Institute for Health Sciences, Rotterdam The Netherlands. In 2009, he achieved the Master. Presently he is Senior Researcher at the National Research Council (C.N.R.) of Reggio Calabria (Italy) and Head of the "Epidemiology and Biostatistics" section of the CNR Research Unit of Reggio Calabria, Italy. His areas of expertise are study design, prospective studies, case-control studies, cross-sectional studies, clinical trial, biostatistics, epidemiology, prognostic biomarkers, diagnostic biomarkers in chronic renal failure, cardiovascular risk. He is Coordinator of the international registry EURECA-m (EUropean RENal and CArdiovasvular Medicine) collecting and merging clinical and epidemiological data, including standard clinical indicators, 24h ambulatory blood pressure monitoring recordings, pulse wave velocity and cardiac imaging of patients with chronic kidney disease treated in Europe.