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Arterial stiffening and vascular calcifications in end-stage renal disease

Alain P. Guérin, Gérard M. London, Sylvain J. Marchais and Fabien Metivier

Service d'Hémodialyse, Hôpital F.H. Manhès, Fleury-Mérogis, France

Abstract

Background. Epidemiological studies have identified aortic stiffness as an independent predictor of cardiovascular mortality in end-stage renal disease (ESRD) patients. In these patients, aortic pulse wave velocity (PWV) was associated with mediacalcosis, but the influence of arterial calcifications on the viscoelastic properties of large arteries was not well characterized. The purpose of the present study was to analyse the influence of arterial calcifications on arterial stiffness in stable haemodialysed patients.

Methods. We studied 120 stable ESRD patients on haemodialysis. All patients underwent B-mode ultrasonography of common carotid artery (CCA), aorta, and femoral arteries to determine CCA distensibility, the elastic incremental modulus (Einc), and the presence of vascular calcifications. All patients underwent measurement of aortic PWV and echocardiogram. The presence of calcifications was analysed semiquantitatively as a score (0 to 4) according to the number of arterial sites with calcifications.

Results. Our observations indicate that arterial and aortic stiffness is significantly influenced by the presence and extent of arterial calcifications. The extent of arterial calcifications is in part responsible for increased left ventricular afterload, and is inversely correlated with stroke volume. The influence of calcifications is independent of the role of ageing and blood pressure. Arterial calcifications density increases with age, duration of haemodialysis, the fibrinogen level, and the prescribed dose of calcium-based phosphate binders.

Conclusions. The results of this study showed that the presence of vascular calcifications in ESRD patients was associated with increased stiffness of large capacity, elastic-type arteries, like the aorta and CCA. The extent of arterial calcifications increased with the use of calcium-based phosphate-binders.

Keywords: arteries; calcifications; calcium carbonate; haemodialysis; viscoelasticity

Correspondence and offprint requests to: Dr Gérard M. London, Hôpital F. H. Manhès, 8 Grande Rue, F-91712 Fleury-Mérogis, Cedex, France.

Introduction

Epidemiological and clinical studies have shown that damage of large arteries is a major contributory factor to the high cardiovascular morbidity and mortality of end-stage renal disease (ESRD) patients [1,2]. The adverse effects of macrovascular disease are attributable to two principal mechanisms: (i) the presence of occlusive lesions, principally atherosclerotic plaques, responsible for ischaemic lesions and/or infarction downstream from the lesion and (ii) stiffening of arterial walls associated with arterial dilatation and hypertrophy [3]. The increased stiffness causes an increase of systolic blood pressure (BP) and pulse pressure and a decrease of diastolic BP, thereby causing increased left ventricular (LV) afterload and altered coronary perfusion [4]. The principal outcomes of these changes are LV hypertrophy, aggravation of coronary ischaemia, and increased fatigue of arterial wall tissues [3–5]. ESRD patients have stiffer arteries than age- and BP-matched non-uraemic subjects [3,6]. Furthermore, in these patients, arterial hypertrophy is accompanied by increased incremental elastic modulus (Einc) and increased pulse-wave velocity (PWV) [3,6,7]. Recent epidemiological studies on ESRD patients have identified aortic PWV and common carotid artery (CCA) Einc as strong independent predictors of their cardiovascular morbidity and mortality [8,9]. While the observation that the Einc is elevated in ESRD patients strongly favours altered intrinsic elastic properties or major architectural abnormalities, the pathogenic factors contributing to arterial stiffening in ESRD are less obvious. Calcification of elastic lamellae and increased calcium contents are observed in the arteries of uraemic patients [10,11]. In haemodialysed patients, aortic PWV was found to be associated with mediacalcosis of the aorta and an elevated calcium $\times$ phosphate product (Ca $\times$ P) [6,12]. Nevertheless, the influence of arterial calcifications and/or factors associated with calcifications on the viscoelastic properties of large conduit arteries in ESRD has not yet been well characterized. The purposes of the present study were to analyse the influence of arterial calcifications on large conduit artery stiffness, and to identify factors associated with arterial calcifications and stiffening.

Subjects and methods

Patients

One hundred and twenty stable, non-diabetic, ESRD patients on haemodialysis for at least 12 months (89 ± 66 months; range: 12–304 months) were included. Based on a clinical work-up and complementary paraclinical investigations (echocardiography, ECG, echo-Doppler examination) patients or controls with acute myocardial infarction, haemodynamically significant valvular heart disease, cerebral vascular disease, CCA stenosis or heart failure were excluded. Patients were dialysed with synthetic membranes (AN69 or polysulphone) and a bicarbonate dialysate with 1.25 or 1.5 mmol/l of calcium according to serum calcium–phosphate equilibrium and the necessity to use 1,25-vitamin D for the control of parathyroid hormone (PTH) activity. The duration of dialysis was individually tailored (4–6 h thrice weekly) to control body fluids and blood chemistries, and to achieve a $Kt/V > 1.2$ (1.46 ± 0.13). Seventy-two patients were regularly receiving recombinant human erythropoietin. Forty-eight patients were taking an antihypertensive drug. Patients regularly took iron and vitamin supplements. Calcium carbonate ($CaCO_3$) was used to maintain predialysis phosphataemia < 2.0 mmol/l. Investigations were performed in the morning before the first weekly haemodialysis session. Each subject gave informed written consent to participate in the study, which was approved by our institutional review board.

Arterial geometry and function

Brachial BP was measured, after 15 min of recumbency with a mercury sphygmomanometer with a cuff adapted to arm circumference. The appearance of Korotkoff sounds was taken as the systolic BP and their disappearance (phase V) as diastolic BP. CCA diameter and wall motion were measured by high-resolution B-mode ultrasonography (Scanner 350, PIE Medical, Maastricht, The Netherlands) with a 7.5-MHz transducer, and echotracking system (Wall-Track System, Maastricht, The Netherlands) enabling assessment of arterial wall displacement during the cardiac cycle. A complete detailed description of this system has been published previously [3]. The radiofrequency signal over six cardiac cycles is digitized and stored in a large memory bank. Two sample volumes, selected under cursor control, are positioned on the anterior and posterior walls. The vessel walls are continuously tracked by sample volumes according to phase and the displacement of the arterial walls is obtained by autocorrelation processing of the Doppler signal. The accuracy of the system is ± 30 μm for CCA diastolic diameter (Dd) and $< \pm 1$ μm for stroke change in CCA diameter (Ds–Dd, where Ds is systolic diameter). The repeatability coefficient of the measurements was ± 0.273 mm for CCA diameter, and ± 0.025 mm for Ds–Dd. Measurements were made on the right CCA, 2 cm beneath the bifurcation. CCA-lumen cross-sectional area (LCSA) was calculated as $\pi(\text{CCA diameter})^2/4$. CCA intima–media thickness (IMT) was measured on the far wall at the same level as the diameter measurements with computer-assisted acquisition, processing, and storage. The computing equipment was linked to an 80386/16 MHz processor and an imaging card providing real-time digitizing of the video analogue signal from the echo-recording (processing corresponding to 256 levels of grey). The IMT was automatically determined from changes of density on the section perpendicular to the vessel wall with specific software (Eurequa, TSA, Meudon, France) [13]. The intima–media cross-sectional area (IMCSA) was calculated as $\pi(\text{CCA diameter}/2 + \text{IMT})^2 - \pi(\text{CCA diameter}/2)^2$, and the wall/lumen ratio as 2IMT/CCA diameter [3]. The repeatability coefficient of the IMT measurement was ± 60 μm [3]. A localized echostructure encroaching into the vessel lumen was considered to be plaque if the CCA IMT was $> 50\%$ thicker than neighbouring sites [14]. Measurements of CCA diameter and CCA IMT were always performed in plaque-free arterial segments in CCA opposite to the site of AV shunts. The presence of plaques was determined in both CCAs.

Diastolic internal aortic diameter was measured ultrasonographically (Sonel 300, Compagnie Générale de Radiologie, Saint-Cloud, France) using 3.5-MHz transducers. The measurements were made blindly by two observers at the level of aortic root diameter and the aortic bifurcation diameter. Interobserver reproducibility was ± 1 mm, their values were then averaged. Aortic PWV was determined as carotid–femoral PWV using the foot-to-foot method [6,15]. Transcutaneous Doppler flow velocity recordings were carried out simultaneously at the base of the neck over the CCA and the femoral arteries in the groin with a SEGA M842 8-MHz Doppler unit (Sociéte d'Electronique Générale et Appliquée, Paris, France) and a Gould 8188 recorder. The time delay (t) was measured between the feet of the flow waves recorded at these different points. The distance travelled by the pulse wave was measured over the body surface as the distance between the two recording sites minus that from the suprasternal notch to the carotid (D). PWV was calculated as $PWV = D/t$. The reproducibility of the measurement has been published previously [16,17].

CCA distensibility was determined from changes of CCA diameter during systole and simultaneously measured CCA pulse pressure (ΔP). CCA pulse pressure was recorded non-invasively by applanation tonometry with a pencil-type probe incorporating a high-fidelity Millar strain gauge transducer (SPT-301, Millar Instruments, Houston, Texas), and sphygmocardiograph (Sphygmocor PWV, Sydney, Australia). The CCA pressure wave was calibrated assuming that brachial and carotid diastolic and mean BPs were equal. Mean BP on the CCA pressure wave was computed from the area of the CCA pressure wave in the corresponding heart period, and set equal to brachial mean BP. CCA pressure amplitude was then computed from diastolic BP and the position of mean BP on the directly recorded CCA pressure wave. A detailed description of this system has been published previously [4,18]. CCA distensibility was determined according to following formulae: $2[(Ds - Dd)/Dd]/\Delta P$ ($kPa^{-1}.10^{-3}$). The repeatability coefficient of the measurement was $\pm 1\ kPa^{-1}.10^{-3}$ for CCA distensibility [4]. While distensibility provides information about the 'elasticity' of the artery as a hollow structure, the Einc gives information on the intrinsic properties of the arterial wall biomaterial. Einc was calculated as $3(1 + \text{LCSA/IMCSA})/\text{CCA distensibility}$ [4].

Echocardiography

All subjects underwent echocardiography with a Hewlett-Packard Sonos 100 device equipped with a 2.25-MHz probe. LV measurements were made according to the recommendations of the American Society of Echocardiography [19]. Measurements were performed blindly by two physicians. Inter- and intraobserver reproducibilities have been reported previously [16,17]. LV mass (LVM) was calculated as $1.05 \times [(PWT + IVST + LVEDD)^3 - (LVEDD)^3] - 13.6$ [20], where PWT is the posterior wall thickness, IVST is the

interventricular septal thickness, and LVEDD is the LV end-diastolic diameter. LV mean wall thickness (LVMWT) was calculated as (IVST + PWT)/2. The fractional shortening of the LV was calculated as [(LVEDD − LVESD)/LVEDD] × 100, where LVESD is the LV end-systolic diameter. Stroke volume was calculated as the aortic valve annular cross-sectional area multiplied by the LV outflow velocity integral, and cardiac output as stroke volume multiplied by heart rate. Total peripheral resistance was calculated as mean BP × 80/cardiac index and expressed in dynes.s.cm^{-5}.m^2. LV diastolic filling was evaluated from pulsed Doppler studies obtained from the apical 4-chamber view of the heart. The sample volume was positioned in the inflow area just at the tip of the mitral leaflets. Maximal early diastolic flow velocity (E) and maximal late atrial flow velocity (A) were measured and their ratio (E/A) calculated.

Arterial calcifications: calcification score

The presence of arterial calcifications was evaluated ultrasonographically in the CCA, the abdominal aorta, the iliofemoral axis, and the legs. Ultrasound examinations were performed by the sonographer at the time of the determination of arterial geometry. The protocol involved scanning of the near and the far walls of CCA(s) in a 4-cm segment preceding the carotid bifurcation. A 10-cm segment of abdominal aorta above its bifurcation was scanned, and the femoral arteries were examined distal to the inguinal ligament proximal to the site of the division of superficial and deep femoral arteries. Arteries were scanned longitudinally and transversely to determine the presence of plaques. A localized echostructure encroaching into the vessel lumen was considered to be plaque when the arterial wall was >50% thicker than neighbouring sites. Highly echogenic plaques producing bright white echoes with shadowing were considered to be calcifications [21]. Assessment of the presence of calcifications was complemented with posteroanterior and lateral fine-detail radiographs of the abdomen and pelvis. Arterial calcifications of the femorotibial arterial axis were evaluated by soft-tissue native radiographs. Arterial calcifications in each arterial region were quantified qualitatively as absent (0) or present (1). The final overall score was obtained by the addition of calcifications from all studied zones. The final score ranged from 0 (absence of calcium deposits), to 4 (calcifications present in all arterial segments examined). The calcification score was independently checked by two observers. The reproducibility was absolute for patients with the score from 2 to 4. Five patients classified as score 0 by one of the observer were classified as score 1 by the second observer. The discordance was related to calcifications of iliofemoral axis which were absent on ultrasonography and visible only after magnification on radiographs. These patients were finaly classified as having score 1.

Blood chemistry

Predialysis blood chemistries determined twice monthly included serum creatinine, urea, calcium, phosphataemia, sodium, potassium, bicarbonates, and haemoglobin. Serum albumin, blood lipids, plasma fibrinogen, C-reactive protein (CRP), and PTH were measured every 3 months. The values considered in the present study are the averages of all above-mentioned measurements over the 1 to 4-year period preceding the study. The occurrence of at least one hypercalcaemic episode (Ca >2.60 mmol/l) was carefully monitored. Total plasma homocysteine was determined by fluorimetric HPLC method [22] and measured only once on the day of haemodynamic evaluation. Smoking habits, prescriptions for calcitriol, and the dose of $CaCO_3$ expressed in grams of elemental calcium prescribed to each patient were recorded from the patients' files.

Statistical analysis

Data are expressed as means ± SD. Patients were classified into five groups according their calcification scores (0–4). Analysis of variance (ANOVA) was used for comparisons of the different groups. Differences in frequency were determined by χ^2 analysis. Single and multiple regression analyses were conducted using the least-squares method and were performed on the entire population. Gender (1, male; 2, female), was used as the dummy variable. Statistical analysis was performed using NCSS 6.0. software (J. I. Hintze, Kaysville, Utah, USA). Repeatability and reproducibility of the methods were defined as recommended by the British Standards Institution [23]. A P value <0.05 was considered significant.

Results

Patient characteristics

Among the BP and cardiac characteristics of the five calcification score groups, only diastolic BP, stroke volume, LV fractional shortening and the E/A ratio differed significantly (Table 1). Diastolic BP was lower in patients with the highest calcification score and negatively correlated with the score ($r = -0.331$, $P < 0.001$). Significant decrease in stroke volume, LV fractional shortening, and E/A ratio of transmitral velocities were observed in subjects with higher calcification score. When clinical parameters and blood chemistries were considered as a function of the calcification score (Table 2), age, smoking habits, the duration of dialysis, lower plasma albumin, increases of CRP and fibrinogen, were significantly associated with higher calcification scores. The other variables evaluated did not appear to be affected except PTH levels which was negatively correlated with calcification score ($P = 0.047$) and age ($P < 0.0001$). Twenty-eight patients presented one or more hypercalcaemic episodes (Ca >2.6 mmol/l), with a significant trend towards more incidences for patients with high calcification scores ($P = 0.034$). Also prescribed dose of $CaCO_3$ increased significantly from group 0 to group 4. All the parameters used to define arterial structures and function were significantly influenced by increasing calcification scores (Table 3). CCA diameter, IMT, aortic root and bifurcation diameters increased progressively with the scores, as did the aortic PWV and CCA Einc. In parallel the CCA distensibility decreased.

Correlation study

The correlation matrix (Table 4) shows the interrelationships between age, blood chemistries, smoking, and duration of dialysis, and calcification scores. Note the age-dependency of many studied parameters and

Table 1. Blood pressure and cardiac parameters according to the calcification score (0 to 4)

Parameters	0 ($n=40$)	1 ($n=15$)	2 ($n=12$)	3 ($n=22$)	4 ($n=31$)	ANOVA
Systolic BP (mmHg)	145±22	150±28	149±39	151±32	151±34	NS
Diastolic BP (mmHg)	88±13	85±11	78±14	77±12	77±15	0.001
Mean BP (mmHg)	108±15	110±15	103±21	101±17	106±17	NS
Pulse pressure (mmHg)	57±14	64±22	70±22	82±28	82±27	0.001
Heart rate (beats/min)	71±11	66±11	66±10	74±13	68±12	NS
Stroke volume (ml)	68±13	74±14	66±24	60±27	55±19	0.01
TPR (dynes.s.cm^{-5}.m^2)	2430±562	2668±912	2524±582	2167±565	2406±695	NS
LV shortening (%)	36±6±6	33±8	38±8	33±7	32±5	0.01
E/A (ratio)	1.06±0.33	1.03±0.29	1.08±0.50	0.80±0.22	0.88±0.33	0.001
LV mass (g)	256±94	288±123	245±51	313±105	295±100	0.07

Values are means±SD. BP, blood pressure; TPR, total peripheral resistance; LV, left ventricle.

Table 2. Clinical characteristics and blood chemistries according to calcification score (0 to 4)

Parameters	0	1	2	3	4	ANOVA
Age (years)	41.4±13.9	48.4±14.2	53.9±18.4	65.7±9.6	63.2±11.7	0.001
M/F ratio	1.4	1.5	1.3	1.45	1.3	NS
Smoking (pack years)	4.8±9.3	11±15.4	5±8.8	17.3±25	17.1±24.4	0.01
BMI (kg/m^2)	22.3±4	24.6±3.8	23.2±2.5	23±3.4	24.6±3.9	NS
Dialysis (months)	52±5	81±67	85±52	101±104	107±86	0.001
Cholesterol (mmol/l)	4.8±1.04	5.17±0.60	4.85±1.25	5.30±1.12	5.05±1.26	NS
HDL (mmol/l)	1.01±0.36	1.16±0.30	1.01±0.19	1.24±0.27	1.09±0.59	NS
Triglycerides (mmol/l)	1.81±0.94	2.07±1.22	2.02±0.70	1.70±0.50	2.03±1.18	NS
Albumin (g/l)	41.9±2.2	40±2.2	40.6±2.27	39.2±2.4	38±2.4	0.001
Fibrinogen (g/l)	3.98±0.77	4.19±0.89	4.26±1.32	4.98±0.74	5.04±1.0	0.001
CRP (mg/l)	5.2±3.6	6.9±3.6	6.4±4.8	11.8±8.3	12.8±11.9	0.001
Homocysteine (mmol/l)	36.1±12.7	38.7±20.1	32.4±12.3	40.5±12.6	31.3±8.3	NS
Haematocrit (%)	32±5.3	29±5.1	31±3	31±3.6	32±4.6	NS
Calcium (mmol/l)	2.30±0.12	2.31±0.18	2.31±0.12	2.35±0.09	2.36±0.12	0.07
Phosphataemia (mmol/l)	1.94±0.43	1.97±0.50	2.0±0.42	1.74±0.40	1.97±0.46	NS
Ca×P (product) (mmol^2/l^2)	4.40±0.80	4.54±1.21	4.57±0.77	4.14±0.83	4.64±1.6	NS
PTH (pg/ml)	360±278	409±332	477±388	221±148	237±285	0.047
$CaCO_3$ (g/day)	1.35±1.10	1.35±0.74	1.50±0.81	1.84±0.94	2.18±0.93	0.001
1,25 (OH)D3 (μg/day)	0.21±0.27	0.28±0.18	0.30±0.49	0.14±0.20	0.21±0.32	NS
% Hypercalcaemia	8	10	18	36	42	0.034

BMI, body mass index; CRP, C-reactive protein; PTH, parathyroid hormone; HDL, high-density lipoprotein; % hypercalcaemia, % of patients having presented at least one episode of calcaemia >2.60 mmol/l.

Table 3. Arterial structure and function according to calcification score (0 to 4)

Parameters	0	1	2	3	4	ANOVA
CCA diameter (mm)	6±0.82	6.3±0.89	6.3±0.70	6.67±0.97	6.73±0.80	0.001
CCA IMT (μm)	700±95	784±156	800±102	849±80	830±81	0.001
Ao_{root} diameter (mm)	26.4±4	26.3±4	27.8±3.9	27.5±4.1	29.7±4.5	0.01
Ao_{bif} diameter (mm)	16±2.4	16.7±4.4	16.7±1.5	17.8±3.3	18.1±2.6	0.01
CCA distens. (kPa^{-1}.10^{-3})	22.6±9.5	17.5±7.8	18.7±11.9	13.5±6.3	11.5±6.3	0.001
CCA Einc (kPa.10^3)	0.47±0.24	0.61±0.31	0.62±0.48	0.76±0.37	1.01±0.60	0.001
Aortic PWV (cm/s)	914±180	946±141	1040±268	1270±384	1302±317	0.001

CCA, common carotid artery; Ao, aortic; Ao_{bif}, aortic bifurcation; CCA distens, CCA distensibility; Einc, incremental elastic modulus; PWV, pulse wave velocity.

the positive correlations between fibrinogen, CRP, and smoking, and the negative correlations between albumin and these parameters. Multiple regression analyses were performed to adjust the roles of different pathogenic factors to age and other confounding variables (Table 5). In univariate analysis the CCA distensibility was negatively correlated with age ($P<0.0001$), blood pressure ($P<0.0001$), calcification score ($P<0.0001$), serum albumin ($P=0.001$), CRP ($P<0.0001$), fibrinogen ($P<0.0001$), and smoking ($P<0.0001$). In univariate analysis, the aortic PWV and CCA Einc were positively correlated with all above-mentioned

Table 4. Correlation matrix between calcification score (Ca score) and variables significantly different between the groups

	Age	Albumin	Smoking	PTH	CRP	Fibrinogen	$CaCO_3$	Dialysis	Ca score
Age	1.00								
Albumin	−0.483**	1.00							
Smoking	0.285*	−0.262*	1.00						
PTH	−0.413**	0.147	−0.193§	1.00					
CRP	0.338**	−0.435**	0.336**	−0.185§	1.00				
Fibrinogen	0.443**	−0.480**	0.361**	−0.162	0.610**	1.00			
$CaCO_3$	0.128	−0.186§	0.095	−0.053	0.255*	0.200§	1.00		
Dialysis	−0.246*	−0.054	−0.150	0.165	0.026	0.010	0.324**	1.00	
Ca score	0.578**	−0.434**	0.285*	−0.204§	0.374**	0.423**	0.396**	0.260*	1.00

§$P<0.05$; *$P<0.01$; **$P<0.001$. PTH, parathyroid hormone; CRP, C-reactive protein; $CaCO_3$, calcium carbonate therapy; dialysis, duration of dialysis in months.

Table 5. Multiple regression analysis between indexes of arterial stiffness and significantly associated variables

Dependent variable Independent variable	β-coeff	t-value	P	Sequential r^2	Partial r^2
CCA distensibility					
Age (years)	−0.333	−8.71	<0.0001	0.411	0.404
Mean BP (mmHg)	−0.262	−8.83	<0.0001	0.636	0.411
Ca score (0–4)	−1.155	−3.00	0.0034	0.671	0.078
	F ratio = 73.5 ($P<0.001$)			adjusted $r^2=0.663$	
CCA Einc					
Age (years)	0.013	5.74	<0.0001	0.278	0.227
Mean BP (mmHg)	0.014	7.63	<0.0001	0.503	0.342
Ca score (0–4)	0.08	3.10	0.0024	0.543	0.080
	F ratio = 44.5 ($P<0.001$)			adjusted $r^2=0.533$	
Aortic PWV					
Age (years)	10.22	6.56	<0.0001	0.385	0.273
Mean BP (mmHg)	6.52	5.40	<0.0001	0.494	0.203
Ca score (0–4)	52.85	3.41	0.0009	0.540	0.092
	F ratio = 43.1 ($P<0.001$)			adjusted $r^2=0.528$	

CCA, common carotid artery; BP, blood pressure; Einc, elastic incremental modulus; PWV, pulse-wave velocity; Ca, calcification score.

Table 6. Multiple regression analysis between arterial calcification score and significantly correlated variables

Dependent variable Independent variable	β-coeff	t-value	P	Sequential r^2	Partial r^2
Calcification score					
Age (years)	0.057	6.53	<0.0001	0.352	0.360
Fibrinogen (g/l)	0.268	2.21	0.0292	0.400	0.042
$CaCO_3$ (g/day)	0.296	2.78	0.0061	0.483	0.067
Dialysis (months)	0.007	5.03	<0.0001	0.580	0.188
	F ratio = 36.67 ($P<0.0001$)			adjusted $r^2=0.565$	

$CaCO_3$ expressed in grams of elemental calcium; dialysis, duration of haemodialysis in months.

parameters. As shown in Table 4, many of these factors are correlated with age and are mutually interrelated. Therefore after adjustment for all these interrelationships, arterial stiffening, whether expressed as CCA distensibility, aortic PWV, or CCA Einc was significantly associated with three factors, i.e. age, mean BP, and the calcification score. After adjustment for all confounding factors, four factors were independently associated with the calcification score: the age, duration of dialysis, dose of $CaCO_3$ prescribed, and plasma fibrinogen concentration. The negative correlation between PTH and calcification score was not significant after adjustment for age. Together, these four parameters accounted for 56.5% of the variance of the calcification score (Table 6).

Discussion

The arterial system of ESRD patients is characterized by dilatation and intima–media hypertrophy of elastic-type, capacitative arteries, such as the aorta or the CCA [3,7]. This remodelling is associated with arterial stiffening [3,6,7]. As the arteries become stiffer, the PWV increases and is responsible for an early return of wave reflections from the periphery to the ascending aorta during systole, causing an abnormal rise of aortic systolic BP and a decrease of diastolic BP [3,4]. This abnormal pressure pattern places an additional load on the LV, leading to LV hypertrophy and altered coronary perfusion [3,4,24,25]. Recent studies have shown that arterial stiffness is a major predictor of all-cause and cardiovascular mortality in haemodialysis patients [8,9].

Arterial stiffness increases with age and hypertension [3,15]. In ESRD patients, the arterial stiffness is increased in comparison to age- and BP-matched non-uraemic subjects [3,6,12,26]. This modification affects elastic- and muscular-type arteries independently of the presence of atherosclerotic plaques [7]. The increase of arterial stiffness is certainly multifactorial, but the exact mechanisms are not clear. In ESRD patients, the arterial stiffening is due to alterations of arterial wall materials as characterized by increased Einc [3,7], and/or major architectural abnormalities like those

seen in experimental uraemia and in the arteries of uraemic patients, namely fibroelastic intimal thickening, calcification of elastic lamellae, increased extracellular matrix with more collagen and relatively less elastic fibre content [10,11]. While the published data agree that smoking is a factor associated with arterial wall hypertrophy in ESRD patients [4,27], no consistent and/or constant associations could be established between arterial remodelling and common vascular risk factors. An association between arterial remodelling and/or functional alterations and lipid abnormalities in ESRD patients were found inconstantly. While London *et al.* [6] and Saito *et al.* [12] reported an inverse relationship between aortic PWV and HDL cholesterol, and Shoji *et al.* [28] reported a positive relationship between aortic PWV and IDL cholesterol, in other studies no correlations were found between serum lipids and arterial changes [3,10,21]. In the present study, we were also unable to establish any correlation between alterations of arterial structure and function, and blood lipid alterations.

The present results demonstrate that plasma fibrinogen level was independently and significantly associated with the extent of arterial calcifications. In addition, fibrinogen concentration was significantly and positively correlated with age, smoking habits and CRP levels, and negatively correlated with serum albumin level. Multiple stepwise regression analysis indicated that fibrinogen was the only biochemical factor significantly associated with the calcification score. Fibrinogen is elevated in ESRD patients [29] and could be associated with endothelial dysfunction and risk factors like smoking, or could merely reflect an inflammatory response as shown by its close association with the CRP level. Several studies have shown that acute-phase reactions, characterized by increased fibrinogen and/or CRP are prominent risk factors for cardiovascular events in the general population as well as in ESRD patients [30,31]. The link between plasma fibrinogen and the presence of arterial plaques and calcifications was also confirmed by Levenson *et al.* in the general population [32].

The factors most frequently quoted in association with arterial stiffening in ESRD patients are altered calcium and phosphate metabolism and parathyroid activity. In haemodialysed patients, aortic PWV was found to be associated with the presence of aortic calcifications and increased $Ca \times P$ products [6,12]. Studying renal transplant recipients, Barenbrock *et al.* [33] observed a relationship between high PTH levels and decreased CCA distensibility. In the present study, we observed a negative association between calcification score and PTH levels, but this correlation was related to the negative correlation between patients' age and PTH level. The present study showed that the presence and extent of arterial calcifications *per se* is associated with arterial stiffening, independently of age and BP (Table 5). The association of calcifications with arterial stiffening was independent of factors such as serum calcium, phosphataemia, $Ca \times P$ product, or PTH concentration, all these factors not being directly or age-independently correlated with arterial stiffness indexes. Arterial calcium contents are increased and arterial calcifications are frequently observed in ESRD patients [10,11]. Cardiovascular calcifications are risk factors for cardiovascular events and, in ESRD patients have been linked to cardiac dysfunction and aortic stenosis [34,35]. Multiple risk factors have been implicated in the development of arterial calcifications. The prevalence of arterial calcifications and calcium content increase with age both in the general population and in ESRD patients [6,10,11]. In ESRD patients, the calcium content is higher than in age-and sex-matched controls [10,11]. In agreement with data reported in the literature [10,11], our findings showed an association between the calcification score and the duration of haemodialysis. Secondary hyperparathyroidism, hyperphosphataemia, elevated $Ca \times P$ products, and increased vitamin D concentrations are frequently evoked as the principal causes associated with vascular remodelling and/or arterial calcifications [36,37]. Kawagishi *et al.* [27] reported that CCA IMT (but not the presence of calcifications) was associated with hyperphosphataemia, while the femoral artery IMT was associated with increased serum PTH. In contrast, Savage *et al.* [21] did not find any relationship between the presence of carotid plaques and PTH levels, and found a negative link between femoral plaque and phosphataemia or $Ca \times P$ products. In their study using electron beam computed tomography, Arad *et al.* [38] did not find the serum concentrations of calcium, 1,25-vitamin D, and PTH to be associated with the presence of arterial calcifications. In the present study the $Ca \times P$ product was lower than the critical value for calcium precipitation (i.e. 5.65 $mmol^2/l^2$), serum calcium was not increased, and phosphataemia was kept at a reasonable level. Moreover, neither phosphataemia nor the $Ca \times P$ product was associated with the calcification score or arterial geometry and stiffness indexes. Patients with higher calcification scores had lower PTH levels, and the only variable that was independently associated with the score of vascular calcifications was the amount of $CaCO_3$ (elemental calcium) prescribed as a phosphate binder. One of the adverse effects of calcium-based phosphate binders is hypercalcaemia, which may in turn result in arterial calcifications. Hyperphosphataemia is frequently observed in patients with low bone metabolic activity and those with adynamic bone disease, and these patients are frequently prescribed higher doses of calcium-based phosphate binders to maintain acceptable phosphate levels. On the other hand, patients with low bone activity and/or adynamic bone disease are more susceptible to develop iatrogenic hypercalcaemia. In the present study, the patients with higher calcification scores and lower PTH concentrations (and probably lower metabolic bone activity) received higher doses of $CaCO_3$ to control phosphataemia and more frequently experienced hypercalcaemic episodes, even if they were transient. PTH levels decreased with age (Table 4) and this decline was coupled with more arterial calcifications. This

picture, associating lower bone activity with arterial calcification, looks like that seen in osteoporosis of the elderly, where the loss of bone calcium and osteopenia is associated with ectopic calcifications in the arteries [39,40]. Osteopenia and reduced bone mass have been found to be associated with stroke, carotid atherosclerosis, and aortic calcifications [41,42]. Growing evidence supports the hypothesis that vascular calcifications develop by mechanisms similar to bone formation and that bone-associated proteins known as Gla-containing proteins, such as osteocalcin and matrix Gla protein, have been localized to advanced atherosclerotic lesions. In addition to these molecules, osteopontin has been also found at sites of arterial calcifications [43,44].

The principal limitation of the present study is the semiquantitative evaluation of vascular calcification. Using the score to evaluate arterial calcification is rather crude and is likely to underestimate the true calcium load. It also does not allow a quantitative assessment of the calcium concentration in the arteries. More sensitive methods (helical or electron-beam computed tomography) should be used in the future to quantify vascular calcification and to assess the time-related alterations of vascular calcium content. However, using a simple score is reproducible and specific, and the method is inexpensive and readily available. The second limitation is represented by the observational nature of the relationship between the calcifications-associated risk of calcium-based phosphate binders. To clarify more precisely the eventual long-term risk associated with the use of these phosphate binders, prospective controlled studies comparing these drugs with alternative ones should be instigated.

In conclusion, the present study shows that the presence of vascular calcifications in ESRD patients is associated with increased stiffness of large, capacitative, elastic-type arteries like the aorta and CCA. The extent of arterial calcification increases with age and the duration of dialysis. The other factors are increased fibrinogen levels, and the use of calcium-based phosphate binders. The use of calcium binders is widely advocated in ESRD patients to replace aluminium salts, but it seems that development of non-calcium binders would be preferable, especially for older patients with lower bone metabolic activity.

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