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Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality

Gérard M. London, Alain P. Guérin, Sylvain J. Marchais, Fabien Métivier, Bruno Pannier and Hasan Adda

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

Abstract

Background. Cross-sectional and follow-up studies on end-stage renal disease patients showed that arterial calcifications are associated with cardiovascular (CV) morbidity and are an independent predictor of all-cause and CV mortality. However, these studies did not examine the impact on prognosis according to the type of calcification, i.e. intimal *vs* medial. Arterial media calcification (AMC), a non-occlusive condition, affects haemodynamics differently from arterial intima calcification (AIC), which occurs in atherosclerotic plaques. The aim of this study was to investigate the prognostic value of AMC in relationship to all-cause or CV mortality for stable haemodialysis (HD) patients.

Methods. We included 202 such patients in the present study. At baseline, soft-tissue native radiograms of the pelvis and the thigh were analysed for the presence and type (AMC *vs* AIC) of arterial calcifications. All patients underwent B-mode ultrasonography of the common carotid artery to determine the presence of atherosclerotic calcified plaques, measurement of aortic pulse wave velocity and echocardiography.

Results. AIC was usually observed in older patients with a clinical history of atherosclerosis before starting HD treatment and typical risk factors associated with atherosclerotic disease. AMC was observed in young and middle-aged patients without conventional atherosclerotic risk factors. AMC was closely associated with the duration of HD and calcium–phosphate disorders, including the oral dose of elemental calcium prescribed as phosphate binder ($CaCO_3$). Compared to patients with AIC, patients with AMC had a longer survival, but in turn their survival was significantly shorter than that of patients without calcifications.

Conclusions. AMC is a strong prognostic marker of all-cause and CV mortality in HD patients, independently of classical atherogenic factors. The principal effect of AMC on arterial function is increased arterial stiffness.

Keywords: arterial calcifications; haemodialysis; mortality

Introduction

Epidemiological and clinical studies have shown that damage of large arteries is a major contributory factor to the high cardiovascular (CV) morbidity and mortality of end-stage renal disease (ESRD) patients [1]. The most prevalent arterial complication is occlusion and/or stiffening caused, to a large part, by increased calcium content and extensive calcifications [2–5]. In the general population and ESRD patients, the presence and extent of arterial calcifications are independently predictive of subsequent CV disease (CVD) and mortality beyond established conventional risk factors [6,7]. Calcification develops at two sites in the arterial wall: the intima and the media. Arterial intima calcification (AIC) represents an advanced stage of atherosclerosis and is associated with the development of plaques and occlusive lesions [8]. Arterial media calcification (AMC), or Mönckeberg's arteriosclerosis, is observed with predilection in muscle-type conduit arteries, such as femoral, tibial and uterine arteries. AMC is commonly associated with aging, presence and duration of diabetes and is common in ESRD [8,9]. Unlike AIC, AMC in its typical form does not obstruct the arterial lumen and was considered to be clinically non-significant [8]. This opinion was challenged by studies on non-insulin-dependent diabetes mellitus (NIDDM), in which AMC presence was a strong marker of future CV risk [9]. In ESRD patients, the extent of arterial calcifications was a strong predictor of all-cause and CV mortality, but no distinction was made between the impact of AIC and AMC, and the pathogenesis and clinical significance of AMC in ESRD

Correspondence and offprint requests to: Dr Gérard M. London, Hôpital F.H. Manhès, 8 Grande Rue, F-91712 Fleury-Mérogis Cédex, France. Email: glondon@club-internet.fr

patients remains uncertain [4,5,7]. The aim of the present study was to assess the clinical significance of AMC in ESRD patients on haemodialysis (HD) and to identify factors associated with the different types of arterial calcifications.

Subjects and methods

Patients

Two-hundred and two ESRD patients on HD for at least 12 months [89 ± 66 months (mean ± SD); range: 12–304 months] were included if they were free of acute myocardial infarction, congestive heart failure and stroke during the 3 months before the inclusion. Patients were dialysed with synthetic membranes and a bicarbonate dialysate with 1.25, 1.5 or 1.75 mmol/l calcium according to the serum calcium–phosphate equilibrium and the necessity to use 1,25-dihydroxy vitamin D3 ($1,25(OH)_2D3$) to control the parathyroid hormone (PTH) levels. The duration of HD 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). Patients received epoietin to maintain pre-dialysis haemoglobin between 100 and 120 g/l. Antihypertensive drugs were prescribed when necessary to maintain post-weekend pre-dialysis blood pressure < 160/90 mmHg. Patients regularly took iron and vitamin supplements. Calcium carbonate ($CaCO_3$) was used to try to maintain pre-dialysis serum phosphate < 2.0 mmol/l. Each subject gave informed written consent to participate in the study, which was approved by our institutional review board.

CV investigations

Brachial blood pressure (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. Common carotid artery (CCA) diameter and intima–media thickness (IMT) were measured by high-resolution B-mode ultrasonography (Scanner 350; PIE Medical, Maastricht, the Netherlands) with a 7.5 MHz transducer and echo-tracking system (Wall-Track System, Maastricht, the Netherlands). A complete detailed description of this system has been published previously [10]. CCA measurements were made 2 cm beneath the bifurcation. CCA IMT was measured on the far wall at the same level as the diameter measurements with computer-assisted acquisition, storage and processing with specific software (Eurequa, TSA, Meudon, France) [10]. A localized echo-structure encroaching into the vessel lumen was considered to be plaque if the CCA IMT was > 50% thicker than neighbouring sites [10]. Measurements of CCA diameter and CCA IMT were always made in plaque-free arterial segments of the CCA opposite to the site of arteriovenous shunts. Plaque presence was determined in both CCA. Diastolic internal aortic diameter at the level of the aortic bifurcation was measured ultrasonographically (Sonel 300; Compagnie Générale de Radiologie, Saint-Cloud, France) using 3.5-MHz transducers. These measurements were made blindly by two observers. Interobserver reproducibility was ± 1mm; their values were then averaged. Aortic pulse wave velocity (PWV) was determined as carotid–femoral PWV using the foot-to-foot method [10,11]. 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:

$$PWV = D/t$$

All subjects underwent echocardiography with a Hewlett-Packard Sonos 100 device equipped with a 2.25 MHz probe. Left ventricular mass (LVM) was calculated as:

$$1.05[(PWT + IVST + LVEDD)^3 - LVEDD^3] - 13.6$$

where PWT is the posterior wall thickness, IVST is the interventricular septal thickness and LVEDD is the LV end-diastolic diameter. LVM was normalized to body height as an index in $g/m^{2.7}$. The fractional shortening of the LV was calculated as:

$$[(LVEDD - LVESD)/LVEDD] \times 100$$

where LVESD is the LV end-systolic diameter. LV diastolic filling was evaluated from pulsed Doppler studies obtained from the apical four-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. All CV measurements were performed during the same session.

Arterial calcifications

Soft-tissue posteroanterior fine-detail native (unenhanced) radiographs of the pelvis and the thigh were taken with patients in the recumbent position. The presence of arterial calcifications was classified as discrete intimal-like plaques with irregular and patchy distribution (AIC) (Figure 1A) or uniform linear railroad track-type in the media (angiogram-like; Figure 1B and C) [9]. The radiological findings were analysed by two observers blinded to clinical data, with interobserver concordance of 92%. Discordances reflected the presence of mixed lesions (Figure 1D) and these patients were classified as AIC ($n = 17$). All patients underwent lower limb Doppler analysis (SEGA M842 8-MHz) to confirm the presence of normally modulated flows and the presence or absence of occlusive lesions was verified in all patients by measuring ankle/arm systolic pressure index (AAI; normal: 0.95–1.30, decreasing with age). In the presence of incompressible arteries and AAI > 1.30, a toe/arm index was obtained. The presence of AMC in muscle-type arteries does not eliminate the possible occurrence of atherosclerosis and plaques in larger elastic-type vessels. Therefore, the presence of arterial plaques and calcifications was evaluated ultrasonographically, as above, in the CCA at the time of arterial geometry determination. X-ray evaluation was done within 1 month of CV tests.

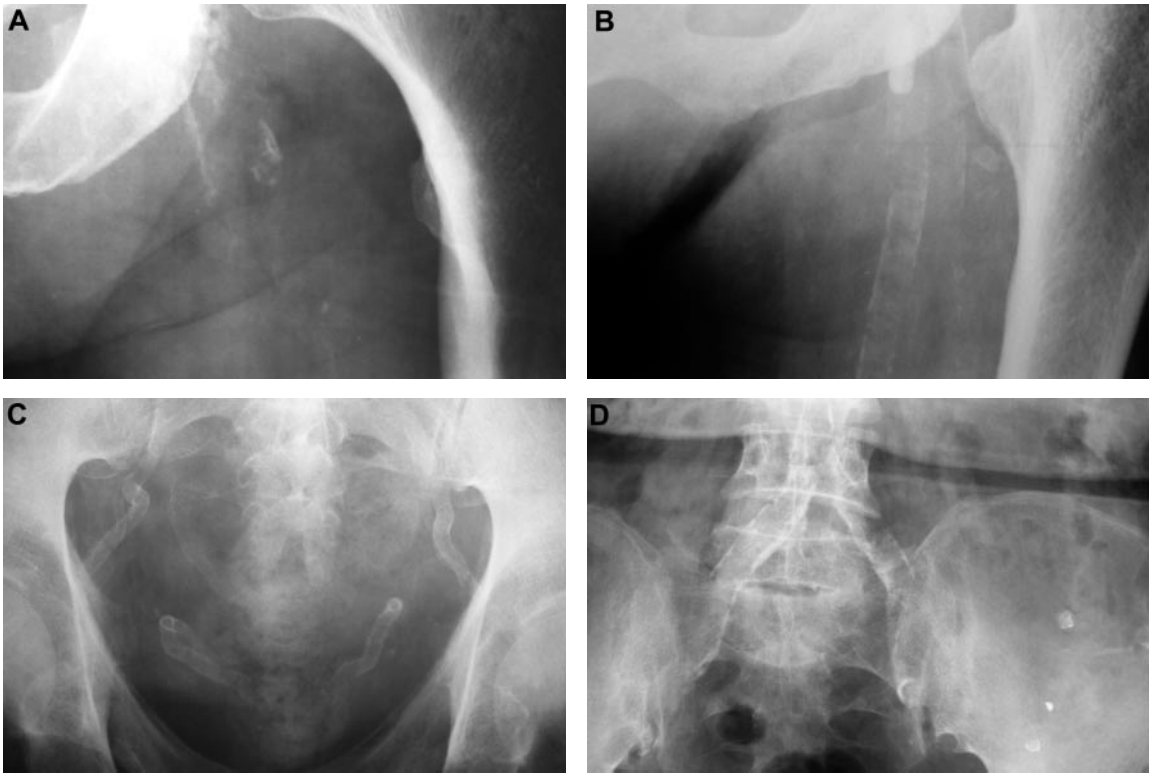

Fig. 1. Calcification of the femoral artery intima (AIC) (**A**) or media (AMC) (**B**). Calcifications of the media of pelvic arteries (AMC) (**C**) and mixed calcifications of the iliac arteries (**D**).

Blood chemistries

Pre-dialysis serum creatinine, urea, calcium, ionized calcium, serum phosphates, sodium, potassium, bicarbonates and haemoglobin were determined twice monthly. 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 year period preceding the study. Total plasma homocysteine was determined with a fluorimetric high-performance liquid chromatography method and measured only once on the day of haemodynamic evaluation. Smoking habits, prescriptions for $1,25(OH)_2D3$ and the $CaCO_3$ dose expressed in grams of elemental calcium/day prescribed to each patient were recorded from the patients' files.

Statistical analyses

Data are expressed as means ± SD unless otherwise specified. Patients were classified into three groups: absence of calcifications (non-calcified, NC), AMC and AIC. Analysis of variance (MANOVA) was used to compare the different groups. Differences in frequencies were determined by chi-square analysis. Gender (0, male; 1, female), diabetes (0, no; 1, yes), race (0, black; 1, white), antihypertensive therapy (0, no; 1, yes), parathyroidectomy (0, no; 1, yes) and type of calcification (0, NC; 1, AMC; 2, AIC) were used as categorial variables. Multinominal logistic regression analyses with NC subjects as the reference group were used to determine the odds ratios (OR) for the type of calcifications associated with clinical and biological parameters. The outcome event studied was all-cause mortality and CV mortality. The primary analyses used the Cox unadjusted model to calculate the risk ratios (RR) associated with different risk factors. Stepwise, multivariate Cox proportional hazard regression was then applied to determine the independent relationship of all unadjusted significant predictors of mortality ($P < 0.05$) with: time variable (follow-up); entry time variable (duration of dialysis before the inclusion); numeric independent variables (age, BP and blood chemistries, including lipids, calcium, phosphate, $CaCO_3$, fibrinogen, albumin, CRP and smoking); and categorial variables (race, history of CV disease, diabetes, parathyroidectomy and type of calcification). The Kaplan–Meier method was applied to estimate survival probabilities and the log-rank test to determine their significance. Variable significance was defined as $P < 0.05$ adjusted for all variables in the final model. All tests were performed using NCSS 7.0 software (J. Hintze, Utah, USA).

Results

Clinical characteristics

The characteristics of the study groups are listed in Table 1. Compared to NC and AMC patients, those with AIC were older at inclusion and at start of HD, had higher prevalence of CVD, diabetes and hypertension/atherosclerosis nephropathy as the principal cause of renal failure. AMC patients had been on HD longer and had a higher proportion of parathyroidectomies than NC subjects, whereas the latter had a significantly higher proportion of blacks.

Table 1. Clinical characteristics of the ESRD patients analysed as a function of arterial calcification status

Variable	NC ($n=73$)	AMC ($n=54$)	AIC ($n=75$)	MANOVA (P-value)
Age at inclusion (years)	45.1 ± 16.3	48.0 ± 13.5	67.0 ± 9.3[a,b]	0.0001
Age at start of dialysis (years)	41.7 ± 17.0	37.5 ± 14.7	62.3 ± 12.0[a,b]	0.0001
Duration of dialysis (months)	43 ± 40	129 ± 74[a,b]	56 ± 62	0.0001
Sex (male/female)	32/41	19/35	34/41	NS
Race (black/white)	23/50	4/50[c]	5/70[a]	0.0025
CVD at start of dialysis (yes/no)	0/73	1/53	55/20[a,b]	0.0001
Smoking (packs/year)	6 ± 13	7 ± 9.0	19 ± 23[a,b]	0.0001
Body mass index (kg/m^2)	22.2 ± 3.6	22.8 ± 3.3	25.2 ± 4.2[a,b]	0.0001
Parathyroidectomy (yes/no)	4/69	23/31[a,b]	9/66	0.00001
Antihypertensive drugs (yes/no)	57/16	35/19	52/23	NS
Chronic glomerulonephritis (%)	60[d]	50[b]	13	0.0001
Chronic interstitial nephritis (%)	18	26	12	NS
Polycystic kidney disease (%)	8	7	8	NS
Diabetes mellitus (%)	3	7	31[a,b]	0.0001
Hypertension/atherosclerosis (%)	7	6	44[a,b]	0.0001
Other (%)	4	3	1	NS

Values are means ± SD. NC *vs* AMC or AIC: [c]$P < 0.05$, [d]$P < 0.01$, [a]$P < 0.001$. AMC *vs* AIC: [b]$P < 0.001$. NS, not significant.

Table 2. Haemodynamic and CV parameters of the ESRD patients as a function of arterial calcification status

Variable	NC	AMC	AIC	MANOVA
Systolic BP (mmHg)	148 ± 24	145 ± 30	154 ± 28	NS
Diastolic BP (mmHg)	87 ± 13	80 ± 14[a]	76 ± 13[b,c]	0.0001
Mean BP (mmHg)	107 ± 16	102 ± 18	102 ± 16	NS
Pulse pressure (mmHg)	60 ± 15	65 ± 20	78 ± 23[b,d]	0.0001
CCA diameter (mm)	7.40 ± 0.8	7.80 ± 0.9[a]	8.60 ± 1.0[b,d]	0.0001
CCA IMT (μm)	721 ± 100	779 ± 108[a]	845 ± 92[b,d]	0.0001
Aortic valve area (cm^2)	2.80 ± 0.8	2.40 ± 0.9[a]	2.0 ± 0.8[b,e]	0.0001
Aortic diameter (mm)	15.8 ± 2.1	16.9 ± 3.3	18.3 ± 3.1[b,e]	0.001
Aortic PWV (cm/s)	921 ± 176	1056 ± 230[b]	1352 ± 309[b,d]	0.0001
Ankle/arm pressure index (ratio)	1.15 ± 0.08	1.12 ± 0.10	0.98 ± 0.25[a,f]	0.01
CCA calcified plaques (%)	4.1	37[b]	90[b,d]	0.0001
LVM index (g/m$^{2.7}$)	74 ± 16	80 ± 19[c]	85 ± 20[a]	0.01
LV % shortening	36.6 ± 6.5	34.0 ± 6.0[c]	32.4 ± 6.1[a]	0.01
Transmitral E/A velocity (ratio)	1.07 ± 0.30	0.98 ± 0.40	0.86 ± 0.38[b]	0.01
Heart rate (beats/min)	70 ± 10	71 ± 11	73 ± 13	NS

NC *vs* AMC or AIC: [c]$P < 0.05$, [a]$P < 0.01$, [b]$P < 0.001$. AMC *vs* AIC: [e]$P < 0.05$, [f]$P < 0.01$, [d]$P < 0.001$. NS, not significant.

The haemodynamic characteristics are listed in Table 2. The values of all parameters usually associated with aging and advanced atherosclerosis and arteriosclerosis, i.e. increased pulse pressure reflecting lower diastolic BP, increased aortic pulse wave velocity, increased CCA diameter and CCA IMT and increased aortic diameter with decreased aortic valve area, changed progressively from NC to AMC to AIC patients. The presence of atherosclerotic plaques in CCA was almost universal in AIC patients and significantly higher in AMC than in NC subjects. The ankle/arm pressure index was similar in NC and AMC patients, but lower in AIC patients. The LV % shortening and E/A ratios decreased from NC to AMC and AIC patients, while LVM index rose.

Blood chemistry results are listed in Table 3. AMC and AIC patients had higher serum CRP, PO_4, $Ca \times PO_4$ product and fibrinogen than NC patients, whose serum albumin was higher. AIC patients had more low-density lipoprotein (LDL) cholesterol in comparison to NC patients and lower PTH than AMC subjects. AMC patients had higher serum Ca than NC and AIC subjects. Finally, higher doses of $CaCO_3$ were prescribed to AMC and AIC patients.

Results of the multinominal logistic regression analyses are shown in Table 4. The OR for the presence of AMC and AIC increased significantly with lower serum albumin, diabetes, time on HD, presence of CCA calcified intimal plaques, white race, serum PO_4 and $CaCO_3$ dose. For AIC patients, OR also increased with age and LDL cholesterol.

Outcome and survival analyses

Causes of death are reported in Table 5. During follow-up, 73 deaths occurred, with 46 due to CV causes. Kaplan–Meier survival curves for the three groups are shown in Figure 2A and B. Non-adjusted and

Table 3. Blood chemistry of the ESRD patients as a function of arterial calcification status

Variable	NC	AMC	AIC	MANOVA
Blood haemoglobin (g/l)	11.2 ± 1.2	11.2 ± 1.3	11.4 ± 1.5	NS
Total serum cholesterol (mmol/l)	4.74 ± 1.05	5.15 ± 1.05	5.23 ± 1.03	0.0161
Serum HDL cholesterol (mmol/l)	1.06 ± 0.40	1.11 ± 0.48	1.06 ± 0.31	NS
Serum LDL cholesterol (mmol/l)	3.92 ± 1.10	4.33 ± 1.12	4.58 ± 1.10	0.0022
Serum triglycerides (mmol/l)	1.44 ± 0.58	1.73 ± 0.70	2.0 ± 0.98	0.0002
Serum albumin (g/l)	40.8 ± 2.8	39.8 ± 2.7[a]	37.9 ± 2.80[b,c]	0.0001
Serum CRP (mg/l)	5.6 ± 6.9	8.9 ± 7.6[b]	15.7 ± 11.6[b,c]	0.0001
Serum PO_4 (mmol/l)	1.72 ± 0.35	1.95 ± 0.45[a]	1.98 ± 0.49[a]	0.0001
Serum calcium (mmol/l)	2.35 ± 0.13	2.40 ± 0.12[d,e]	2.35 ± 0.12	0.0100
Serum ionized calcium (mmol/l)	1.22 ± 0.08	1.23 ± 0.08	1.17 ± 0.07[e,b]	0.0001
Serum $Ca \times PO_4$ ($mmol^2/l^2$)	3.96 ± 0.98	4.76 ± 1.01[b]	4.60 ± 1.04[b]	0.0001
Serum homocysteine (μmol/l)	33.3 ± 13.8	37.5 ± 14.7	35.4 ± 10.6	NS
Serum fibrinogen (g/l)	3.67 ± 0.78	4.37 ± 0.88[b]	4.73 ± 0.98[b]	0.0001
PTH (pg/ml)	320 ± 270	390 ± 318[d]	254 ± 271	0.0340
Dose of $CaCO_3$ (g elemental Ca/day)	1.10 ± 0.95	2.20 ± 1.00[b]	2.0 ± 1.10[a]	0.0001
Dose of $1,25(OH)_2D3$ (μg/day)	0.20 ± 0.24	0.26 ± 0.33	0.16 ± 0.27	NS

NC *vs* AMC or AIC: [e]$P < 0.05$, [a]$P < 0.01$, [b]$P < 0.001$. AMC *vs* AIC: [d]$P < 0.05$, [c]$P < 0.01$. NS, not significant.

Table 4. Multinominal adjusted logistic regression report of parameter significance with the NC group as the reference value

Parameter	Group	Wald *Z*-value	*P*-value	OR (95% CI)
Age (years)	AMC	0.420	0.6747	1.00 (0.94–1.04)
	AIC	2.096	0.0361	1.06 (1.00–1.12)
Serum albumin (g/l)	AMC	–4.780	0.0000	0.73 (0.64–0.83)
	AIC	–5.351	0.0000	0.68 (0.59–0.78)
Diabetes (0, no; 1, yes)	AMC	2.246	0.0246	30.8 (1.55–612)
	AIC	2.359	0.0183	48.9 (1.93–1240)
CCA plaques (0, no; 1, yes)	AMC	2.812	0.0049	13.6 (2.20–84.8)
	AIC	5.902	0.0000	227.4 (37.5–1379)
Race (0, black; 1, white)	AMC	2.361	0.0182	9.42 (1.46–60.67)
	AIC	2.308	0.0210	16.8 (1.53–186)
Duration of dialysis (months)	AMC	4.766	0.0000	1.03 (1.02–1.05)
	AIC	2.615	0.0089	1.02 (1.01–1.04)
Serum PO_4 (mmol/l)	AMC	2.664	0.0077	12.93 (1.97–85.0)
	AIC	2.668	0.0076	15.6 (2.07–117.6)
Dose of $CaCO_3$ (g elemental Ca/day)	AMC	3.114	0.0019	2.55 (1.41–4.59)
	AIC	2.058	0.0396	2.06 (1.04–4.10)
Serum LDL cholesterol (mmol/l)	AMC	1.261	0.2074	1.42 (0.82–2.46)
	AIC	2.657	0.0079	2.22 (1.23–4.02)

Model $r^2 = 0.601$; $P < 0.00001$. CI, confidence interval.

Table 5. Causes of death during follow-up of ESRD patients as a function of arterial calcification status

Event	NC	AMC	AIC	*P*-value
All-cause death	5	23[a]	45[a,b]	0.0001
CV death	2	12[a]	32[a,b]	0.0001
Myocardial infarction	0	3[c]	23[a,d]	0.0001
Heart failure	2	2	7	NS
Stroke	0	1	2	NS
Sudden death	0	6[e]	0[b]	0.005

[a]$P < 0.001$ AMC and/or AIC vs NC; [b]$P < 0.05$ AMC *vs* AIC; [c]$P < 0.05$ AMC *vs* AIC; [d]$P < 0.01$ AMC *vs* AIC; [e]$P < 0.01$ AMC *vs* NC.

multivariate Cox regression reports for all-cause and CV mortality are shown in Tables 6 and 7. All-cause mortality was independently and significantly associated with the type of calcification: RR for AMC *vs* NC group was 15.7 (4.8–51.4; $P < 0.00001$) and for AIC *vs* NC group was 4.85 (1.68–14.10; $P = 0.0036$). The other factors associated with all-cause mortality were diabetes, serum calcium, CRP, previous parathyroidectomy and pulse pressure as the haemodynamic consequence of calcification-associated stiffness

of arteries (Table 6). Similar factors with the exception of diabetes and parathyroidectomy were associated with CV death (Table 7).

Subgroup analyses. Although the Cox regression analyses did not indicate that HD duration had an impact on outcome, the difference of HD durations between NC and AMC patients could introduce a bias into the analyses. The same holds true for the higher frequency of calcified atherosclerotic intimal plaques in the CCA of AMC patients than of NC patients. For these reasons, subgroup analyses were done between NC and AMC patients without carotid plaques and these were further matched for HD duration (Table 8). In comparison to NC subjects, all-cause (Figure 3A) and CV survival (data not shown) were significantly shorter in AMC patients, even in the absence of atherosclerotic plaques. The same results, i.e. shorter CV and all-cause survival, were obtained for AMC patients matched for HD duration (Figure 3B). The main difference between these two groups was the significantly higher proportion of blacks in NC patients. Serum Ca, serum PO_4 and $Ca \times PO_4$ were higher in AMC patients for whom higher $CaCO_3$ doses were prescribed. The principal haemodynamic differences between AMC and NC patients were higher aortic PWV and lower diastolic pressure for the former.

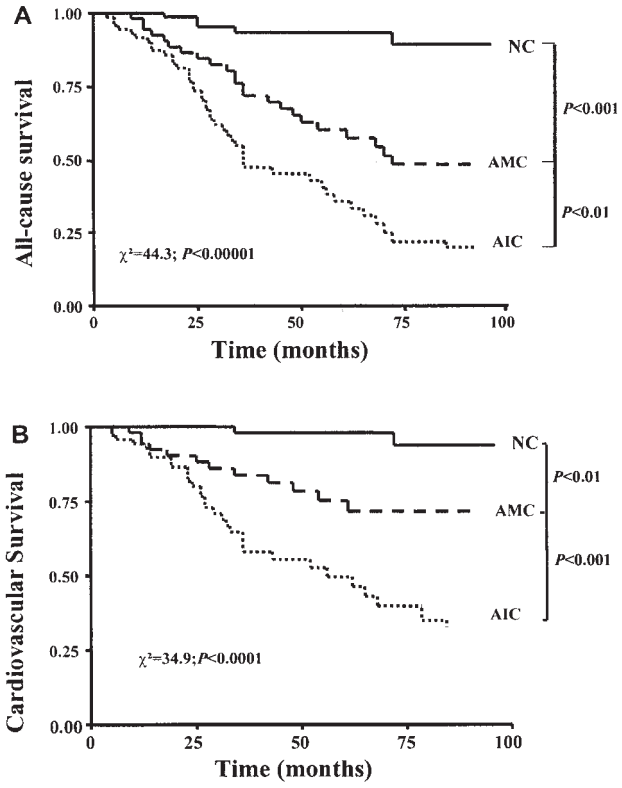

Fig. 2. All-cause (**A**) and CV mortality (**B**) of ESRD patients as a function of their arterial calcification status.

Discussion

The results of this study confirm that the presence of arterial calcifications in ESRD patients is strongly predictive of outcome and demonstrates that AMC is a powerful and independent prognostic marker for all-cause and CV mortality in chronic HD patients.

Arterial calcifications develop at two sites in the arterial wall: the intima and the media [8]. As the present results show, AIC developed predominantly in older individuals and was associated with a clinical history of diabetes and atherosclerosis and atherosclerotic complications, including vascular nephropathy, the almost

Table 6. Cox regression report for all-cause mortality

Variable	Wald *Z*-value	*P*-value	RR (95% CI)
Unadjusted			
Age (years)	3.61	0.0003	1.03 (1.01–1.04)
Pulse pressure (mmHg)	5.17	0.0000	1.03 (1.01–1.04)
Diabetes (yes, no)	3.05	0.0023	3.33 (1.39–4.76)
Serum albumin (g/l)	–3.38	0.0007	0.95 (0.92–0.98)
Fibrinogen (g/l)	5.30	0.0000	1.97 (1.53–2.54)
CRP (ln mg/l)	6.26	0.0000	2.98 (2.12–4.19)
Serum calcium (mmol/l)	2.63	0.0084	17.0 (2.06–139.5)
$CaCO_3$ (g elemental Ca/day)	6.02	0.0000	1.92 (1.55–2.38)
$Ca \times PO_4$ ($mmol^2/l^2$)	2.27	0.0232	1.28 (1.03–1.58)
AMC (yes, no)[a]	7.70	0.0000	46.7 (17.6–124.2)
AIC (yes, no)[a]	5.75	0.0000	14.9 (5.9–37.7)
Parathyroidectomy (yes, no)	6.46	0.0000	6.25 (3.57–11.1)
Adjusted			
AMC (0, no; 1, yes)[a]	4.55	0.0000	15.7 (4.8–51.4)
AIC (0, no; 1, yes)[a]	2.91	0.0036	4.85 (1.68–14.0)
Diabetes (0, no; 1, yes)	3.12	0.0018	2.66 (1.44–4.92)
Serum calcium (mmol/l)	2.74	0.0062	20.6 (2.36–180.0)
CRP (Ln mg/l)	2.20	0.0276	1.67 (1.06–2.63)
Pulse pressure (mmHg)	3.20	0.0014	1.02 (1.01–1.03)
Parathyroidectomy (0, no; 1, yes)	2.38	0.0173	3.23 (1.23–8.33)

[a]NC group as the reference. CI, confidence interval.

Table 7. Cox regression report for CV mortality

Variable	Wald *Z*-value	*P*-value	RR (95%CI)
Unadjusted			
Age (years)	3.07	0.0022	1.03 (1.01–1.05)
Pulse pressure (mmHg)	4.26	0.0000	1.03 (1.01–1.04)
Diabetes (0, no; 1, yes)	2.47	0.0135	2.56 (1.20–5.26)
Serum albumin (g/l)	–2.66	0.0079	0.95 (0.92–0.99)
Serum phosphate (mmol/l)	2.58	0.0098	2.19 (1.21–3.97)
CRP (ln mg/l)	5.44	0.0000	3.50 (2.23–5.21)
Serum calcium (mmol/l)	2.79	0.0053	48.2 (3.17–747.0)
$CaCO_3$ (g elemental Ca/day)	5.77	0.0000	2.23 (1.70–2.94)
AMC (0, no; 1, yes)[a]	4.38	0.0000	60.9 (13.5–276.0)
AIC (0, no; 1, yes)[a]	5.33	0.0000	24.7 (5.90–103.9)
History of CVD (0, no; 1, yes)	5.14	0.0000	5.26 (2.78–9.01)
Parathyroidectomy (0, no; 1, yes)	4.27	0.0000	5.02 (2.38–9.00)
Adjusted			
AMC (0, no; 1, yes)[a]	4.85	0.0000	45.7 (9.75–213.0)
AIC (0, no; 1, yes)[a]	2.54	0.0110	7.50 (1.59–35.5)
Serum calcium (mmol/l)	2.10	0.0366	17.4 (1.20–253.0)
CRP (Ln mg/l)	1.97	0.0427	1.77 (1.02–3.06)
Pulse pressure (mmHg)	2.37	0.0178	1.02 (1.01–1.04)

[a]NC group as the reference. CI, confidence interval.

Table 8. Clinical characteristics of NC patients and patients with AMC, matched for the duration of HD and exclusion of diabetics

Variable	NC ($n=30$)	AMC ($n=30$)	*P*-value
Age at start of dialysis (years)	35 ± 17	37 ± 14	NS
Duration of HD (months)	74 ± 33	74 ± 38	NS
Sex (male/female)	17/13	20/10	NS
Race (black/white)	12/18	3/27	0.0025
Systolic BP (mmHg)	151 ± 22	151 ± 30	NS
Diastolic BP (mmHg)	89 ± 11	85 ± 11	0.0151
Smoking (packs/year)	6 ± 7	8 ± 11	NS
Body mass index (kg/m^2)	22 ± 3.30	22.6 ± 3.20	NS
Aortic PWV (m/s)	974 ± 193	1101 ± 210	0.0173
Total cholesterol (mmol/l)	4.66 ± 1.0	5.00 ± 0.96	NS
Serum albumin (g/l)	41.7 ± 2.80	40.1 ± 2.70	0.0150
Serum CRP (mg/l)	5.10 ± 5.40	8.5 ± 6.4	0.0291
Serum calcium (mmol/l)	2.33 ± 0.12	2.40 ± 0.12	0.0246
Serum PO_4 (mmol/l)	1.75 ± 0.34	2.05 ± 0.41	0.0013
Serum $Ca \times PO_4$ ($mmol^2/l^2$)	4.10 ± 0.89	4.92 ± 1.00	0.0005
Dose of $CaCO_3$ (g elemental Ca/day)	1.10 ± 0.95	2.15 ± 1.00	0.0003
Dose of $1,25(OH)_2D_3$ (μg/week)	0.30 ± 0.30	0.15 ± 0.30	NS
PTH (pg/ml)	369 ± 307	378 ± 316	NS

NS, not significant.

universal presence of calcified plaques in CCA, lower ankle/arm pressure index, enlargement-hypertrophy of the aorta and CCA, a longer history of smoking and higher LDL cholesterol and serum CRP levels. In addition to these non-specific risk factors, AIC were also associated with ESRD-specific risks, such as elevated serum PO_4, lower serum albumin and higher $CaCO_3$ intake. The logistic regression analyses indicated that the OR for AIC presence increases also with HD duration. The outcome for AIC patients was worst, with higher rates of myocardial infarction and arterial complications (Table 5).

While AIC is apparently associated with generalized atherosclerosis, which is not specifically attributable to HD, AMC seems to be much more closely associated with HD treatment and its duration. Patients with AMC are much younger than those with AIC. Although they were the same age as NC patients, they had been on HD for much longer. The origin of the nephropathies also differed with more primary renal diseases, less diabetes and less hypertensive-vascular diseases and absence of clinical history of CVD at start of HD therapy for NC and AMC patients than AIC subjects. Nevertheless, in comparison with NC subjects, AMC patients developed several CV complications, some of which could be attributed to atherosclerosis and their intimal calcifications. Indeed, even in the typical form of AMC in femoral, tibial or uterine arteries, AIC could coexist and an atherosclerotic plaque burden could be found in the larger elastic-type

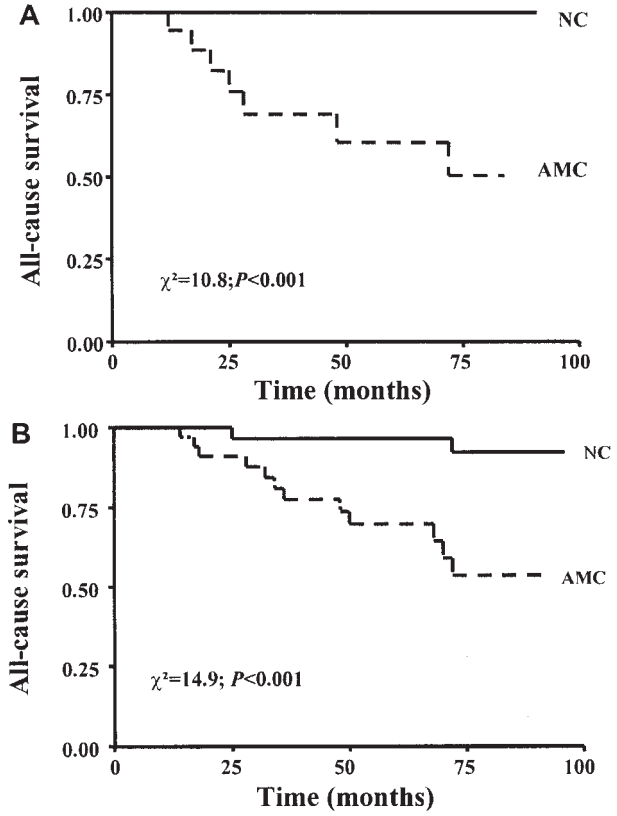

Fig. 3. All-cause mortality of NC and AMC ESRD patients matched for duration of HD at inclusion into the follow-up (**A**). NC and AMC patients free of common carotid artery calcified plaques (**B**).

arteries; 37% of AMC patients had calcified CCA intimal plaques (*vs* 90% of AIC patients) and increased CCA IMT (the extent of carotid atherosclerosis correlates positively with the severity of coronary atherosclerosis and major coronary events [12]), indicating that AMC patients also developed atherosclerotic lesions. To minimize the role of these lesions in survival, we compared subgroups of patients with NC or AMC and normal CCA. As shown in Figure 3A , the survival of AMC patients was significantly shorter than that of NC patients, emphasizing the role of AMC alone. Finally, while the Cox regression analyses did not retain HD duration as a prognostic factor, the fact that AMC patients had been on HD for a significantly longer time could introduce a selection bias. For this reason, we compared in a second analysis subgroups of AMC and NC patients matched for HD duration of dialysis, with the exclusion of diabetic patients. The results again indicated that AMC were significantly associated with poor survival (Figure 3B).

In the past, it was considered that vascular calcification was a passive degenerative process, but recent data have shown that vessel wall calcification is an actively regulated process sharing similarities with osteogenesis, with the expression of many bone and mineralization regulatory factors in calcified lesions [8,13,14]. The expression of these factors is not specific to the uraemic state and the role of uraemia as a factor favouring soft-tissue calcification appears to be the consequence of an electrolyte imbalance, mainly a perturbed phosphate–calcium homeostasis [15]. Increased serum PO_4 and a high $Ca \times PO_4$ product favour mineral deposition on the elastin components of the vascular matrix. Moreover, a high PO_4 concentration upregulates Cbfa1, the factor that induces the transformation of vascular smooth muscle cells to an osteoblastic phenotype [14]. As the present study shows, the serum PO_4 and $Ca \times PO_4$ product was higher in patients with calcifications than in NC patients. Logistic regression indicates that the RR for the presence of AMC is associated with higher serum PO_4. The possibility that the oral calcium dose prescribed to patients favours vascular calcification is supported by a recent study by Chertow *et al.* [16], who demonstrated that progression of arterial calcification scores for the aorta and coronary arteries was more rapid in patients receiving calcium-containing phosphate binders than in those taking sevelamer. No role could be attributed to PTH based on our findings. Serum PTH levels were higher in AMC patients than AIC patients (but not NC subjects), but significantly more AMC patients had undergone parathyroidectomy, which may introduce a serious bias in the analyses concerning PTH. The logistic regression analysis did not indicate any relationship between PTH and calcifications, in agreement with Arad *et al.* [17]. While animal and *in vitro* studies demonstrated that vitamin D and $1,25(OH)_2D3$ increase vascular calcifications, clinical studies in non-uraemic subjects unexpectedly showed that there was an inverse or no relationship between serum $1,25(OH)_2D3$ and vascular calcifications [18].

Our findings suggest that abnormal calcium–phosphate metabolism is not the only factor associated with AMC, as indicated by the significantly lower prevalence of calcifications in black subjects. Racial differences in coronary calcifications and calcium content, with lower rates in blacks than in white patients, have been demonstrated [5]. Evidence for some degree of heritability of coronary artery calcification and calcium deposits in the aorta has also been obtained, suggesting the influence of genetic factors in the calcification process [19].

Lower serum albumin and higher serum CRP were associated with higher OR for the presence of AIC and AMC, thereby suggesting that chronic low-grade inflammation and/or malnutrition favour the occurrence of both types of calcifications in ESRD. Low-grade inflammation and lower serum albumin are typical findings in ESRD patients, but the association of CV calcifications and inflammation is not restricted to uraemic patients since it has also been observed in non-uraemic subjects [20]. The OR for the presence of AIC was associated with LDL cholesterol, in agreement with previously suggested interactions between lipids and CV calcification [21].

The prognosis of ESRD patients is significantly influenced by the presence of calcifications. The higher mortality of AIC patients could be explained by the

high rate of occlusive lesions and generalized atherosclerosis. However, the discussion remains concerning the relationship between calcifications and plaque stability. According to some authors, calcification does not decrease mechanical stability of plaques [22]. The problem is that the highly heterogeneous constituents of atherosclerotic plaques exhibit a broad spectrum of bilinear mechanical properties, and strain and stress energy distributions, and consequently the sliding forces are substantially increased at the interface between rigid calcified tissues and the distensible components within the plaque [23]. Besides the possible role of associated plaques and occlusive lesions (37% of AMC patients had CCA calcified plaques), the second reason for the higher mortality of AMC patients is arterial stiffening, characterized by increased aortic PWV. Several studies in ESRD, essential hypertension and NIDDM patients have shown that arterial stiffening is an independent predictor of mortality [2,24]. As the arteries become stiffer, the PWV increases and is responsible for an earlier return of wave reflections from the periphery to the ascending aorta during systole, which causes an abnormal rise of aortic systolic BP with decreased diastolic BP and high pulse pressure. Increased wave reflections and high pulse pressure are independent risk factors for mortality of ESRD patients [25].

An important limitation of the present study is the qualitative evaluation of the calcifications with the use of native soft-tissue radiographs. Intimal and medial forms are usually distinguished easily with good interobserver's concordance, but the intensity of calcification in terms of the quantitative calcium content per gram of tissue cannot be determined with this technique. However, the method is readily available and relatively specific. Moreover, it allows the distinction between intimal and medial calcifications, which is not presently possible with other techniques. AMC can be associated with atherosclerosis and intimal plaques and occlusive lesions and their detection must always lead to Doppler analysis of flow waves and ankle/arm pressure index to eliminate the presence of occlusive lesions and subclinical atherosclerosis.

In conclusion, AMC, as visualized by plain soft-tissue radiograms, is significantly associated with all-cause and CV mortality of ESRD patients. AMC is significantly less frequent in blacks and its extent increases with HD duration. Factors associated with AMC are diabetes, high serum PO_4, high doses of calcium-containing phosphate binders and low serum albumin (and higher CRP). Screening for vascular calcifications by conventional X-ray could be an easily accessible method to screen dialysis patients for their individual CV risk. Interventional studies are needed to evaluate the respective roles of these factors and to define and optimize future therapeutic strategies.

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