Arterial Calcifications and Bone Histomorphometry in End-Stage Renal Disease

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Abstract. Arterial calcification (AC) is a common complication of end-stage renal disease (ESRD). The mechanisms responsible are complex, including disturbances of mineral metabolism and active expression of various mineral-regulating proteins. An inverse relationship between AC and bone density has been documented in uremic patients. In the study presented here, which included 58 patients with ESRD on hemodialysis (HD), bone-histomorphometry characteristics were compared with the AC scores (0 to 4) determined according to the number of arterial sites with calcifications. Patients with AC scores of 0 (no calcifications), or 1 or 2 (mild calcifications) had similar serum parathyroid hormone levels and bone histomorphometry, with larger osteoclast resorption, higher osteoclast numbers, and larger osteoblastic and double tertracycline-labeled surfaces. In contrast, patients with high AC scores (3 and 4) were characterized by lower serum parathyroid hormone, low osteoclast numbers and osteoblastic surfaces, smaller or absent double tetracycline-labeled surfaces, and high percentages of aluminum-stained surfaces. According to multivariate analysis, AC score was positively associated with age ($P < 0.0001$), daily dose of calcium-containing phosphate binders ($P = 0.009$), and bone aluminum-stained surfaces ($P = 0.037$), and an inverse correlation was observed with osteoblastic surfaces ($P = 0.001$). A high AC score is associated with bone histomorphometry suggestive of low bone activity and adynamic bone disease. These findings suggest that therapeutic interventions associated with excessive lowering of parathyroid activity (parathyroidectomy, excessive calcium or aluminum load) favor lower bone turnover and adynamic bone disease, which could influence the development and progression of AC.

Arterial calcification (AC) is a common complication of end-stage renal disease (ESRD) (1–3). In the general population and patients with ESRD, the extents of AC were predictive of subsequent cardiovascular disease and mortality beyond established conventional risk factors (4–6). The mechanisms responsible are complex, but abnormalities in mineral and phosphate metabolism in particular are thought to be important determinants (7). For many years, AC was considered to be the result of passive mechanisms due to elevated phosphate levels and high calcium-phosphate ion product resulting in supersaturated plasma (7–9). However, recent studies have shown that AC is a regulated process with plasma constituents maintaining minerals in solution and inhibiting their deposition in tissues (10). In addition, evidence indicates that many proteins involved in bone metabolism can be expressed in arterial tissue, reflecting changes in the phenotype of vascular smooth-muscle cells (11–17). Disturbances in calcium (Ca) and phosphate (PO_4) metabolism in ESRD are associated with changes of parathyroid hormone (PTH) secretion and uremic bone disease, but their role in the pathogenesis of AC remains uncertain (1,2,18,19). An inverse relationship between AC and bone density has been documented in uremic patients (20). In the general population, AC and osteoporosis are also associated, and a relationship exists between the clinical course of the two processes (21). Treatment with PTH increases bone strength and bone mineral density in postmenopausal osteoporosis (22,23). The study presented here was designed to determine whether the alterations of bone activity, as assessed by bone histomorphometry, are associated with the extent of AC in patients with ESRD undergoing chronic hemodialysis (HD).

Materials and Methods

Patients

Fifty-eight white nondiabetic patients with ESRD on HD for at least 12 mo (median, 126 mo; range, 12 **to** 304 mo) were included. Bicarbonate dialysate was prepared by reverse osmosis–treated water with 1.25, 1.5, or 1.75 mmol/L of Ca, according to the serum Ca-PO_4 equilibrium and the need to use vitamin D_3 and calcium carbonate ($CaCO_3$). Although $CaCO_3$ was exclusively used as a PO_4 binder at the time of the study, 33 patients had taken aluminum hydroxide ($Al(OH)_3$) in the past. Twenty-three patients underwent subtotal parathyroidectomy (PTX), with heterotopic autotransplantation into the forearm in 10 of them. PTX was performed 20 to 70 mo before the study. The duration of HD was individually tailored (4 **to** 6 h three times a week) to control body fluids and blood chemistries, and to achieve a Kt/V > 1.2 (1.46 ± 0.13). Patients regularly took iron and vitamin supplements.

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AC Score

The presence of AC was evaluated ultrasonographically in the common carotid arteries, the abdominal aorta, the iliofemoral axis, and the legs, as described previously (2). The protocol involved scanning of the near and far walls of the common carotid arteries and abdominal aorta. Femoral arteries were examined distal to inguinal ligament and proximal to the site of the division of superficial and deep femoral arteries. Arteries were scanned longitudinally and transversely to determine the echogenic presence of plaques. A localized echogenic structure encroaching into the vessel lumen was considered to be plaque when the arterial wall was >50% thicker than neighboring sites (2). Highly echogenic plaques producing bright white echoes with shadowing were considered to be calcifications. Assessment of the presence of calcifications was completed with lateral fine-detail radiographs of the abdomen, posteroanterior radiographs of the pelvis, and calcifications of the femorotibial axis by unenhanced soft tissue radiographs. AC in specific regions were evaluated by a binary notation as absent (0) or present (1). The final overall score, obtained by adding the AC scores from all studied regions, ranged from 0 (no visible calcium deposits) to 4 (generalized calcifications present in all arterial segments examined). The AC score was independently checked by two observers blinded to clinical data, with interobserver concordance of 95% (2,6). AC score assessment and bone histomorphometry were done within a period of 3 mo.

Bone Histomorphometry

Diagnostic anterior iliac crest bone biopsies were performed after double tetracycline labeling according to the schedule of 2 d on tetracycline, 10 d off, and 2 d on (24). On three 5-(m-thick sections stained with toluidine blue, trabecular bone volume (%), osteoid surface and volume (%), osteoblast surface (%), osteoclast resorption surface (%), and osteoclast number (/mm^2) were assessed. On two unstained 10-(m-thick sections, the bone mineralization rate and the extent of double and total tetracycline-labeled surfaces (%) were evaluated. Bone aluminum (Al) staining was evaluated according to the method described by Maloney *et al.* (25) and expressed as the percentage of the trabecular surface stained. All measurements were made with an eyepiece reticule (Zeiss integral plate II, Oberkochen, FRG).

Blood Chemistries

Blood chemistries including serum Ca and PO_4, blood lipids, intact PTH (1 **to** 84), serum 25(OH)D_3, and serum Al were determined the week before bone biopsy. Serum Al was measured with atomic absorption spectrophotometry and graphite furnace. Plasma samples for quantification of the Al concentration were obtained before routine HD and 40 h after infusion of deferoxamine (DFO). DFO was perfused over a 2-h period at a rate of 40 mg kg body wt immediately after HD. Smoking habits (20 patients were past smokers and 3 still smoked), prescriptions for vitamin D_3 (μg/d), and the $CaCO_3$ dose expressed in grams of elemental Ca per d prescribed to each patient were recorded from the patients' files. All subjects gave informed written consent to participate in the study, which was approved by our institutional review board.

Statistical Analyses

Data are expressed as medians and ranges. The primary analysis concerning the overall population. The secondary analysis concerned patient subgroups (*i.e.*, PTX *versus* no PTX, and positive *versus* negative bone Al staining). Analysis was performed by Kruskal-Wallis one-way ANOVA on ranks; Kruskal-Wallis multicomparison

Table 1. Clinical characteristics of the overall ESRD population analyzed as a function of the arterial calcification scores[a]

Variable	0 $n = 9$		1 $n = 10$		2 $n = 12$		3 $n = 11$		4 $n = 16$		KW (P value)	Trend (P value)	Group Differences
Age (yr)	33	(24–60)	37.5	(23–57)	56	(40–65)	58	(41–70)	62	(52–75)	0.0001	0.0001	0,1 *versus* 2–4
Duration of HD (mo)	120	(18–156)	120	(12–204)	144	(12–304)	179	(32–296)	102	(12–296)	0.32	–	–
Smoking (pack-yr)	0	(0–18)	2	(0–15)	1	(0–51)	8	(0–32)	14	(0–35)	0.09	–	–
PTX (yes/no)		1/9		1/10		3/12		8/11		10/16	0.0151 (χ^2)	–	0–2 *versus* 3,4
Systolic BP (mmHg)	162	(103–193)	145	(99–193)	158	(98–198)	155	(106–190)	167	(90–186)	0.37	–	–
Diastolic BP (mmHg)	98	(64–108)	85	(48–108)	85	(60–100)	77	(60–100)	78	(55–96)	0.24	–	–
Cholesterol (mmol/L)	4.44	(2.1–6.77)	4.86	(3.32–6.89)	4.80	(2.45–7.95)	5.80	(3.13–7.75)	5.25	(3.9–6.13)	0.12	–	–
Serum albumin (g/L)	41.2	(38.0–44.1)	40.9	(38.6–43.0)	39.3	(37.0–40.3)	39.4	(33.1–41.4)	37	(34.0–40.0)	0.002	0.0001	0–1 *versus* 4
Serum CRP (mg/L)	2.5	(1.0–8.5)	3.0	(2.0–5.5)	6.5	(1.0–38.0)	10.3	(6.0–30.0)	11	(6.5–29.0)	0.001	0.001	3,4 *versus* 0,1

[a] KW, Kruskal Wallis; HD, hemodialysis; BP, blood pressure; PTX, parathyroidectomy; CRP, C reactive protein.

Z-value test and Tukey-Kramer multiple comparison test were used to compare the different AC scores. Univariate and multivariate regression analyses were performed by the least-squares method. Gender (0, male; 1, female) was used as a categorical variable. All tests were performed by NCSS version 7.0 (J. Hintze, Kaysville, UT). $P < 0.05$ was considered significant.

Results

All Patients

Clinical Characteristics, Blood Chemistries, and Bone Histomorphometry. Among the characteristics of the study patients as a function of their AC scores (Tables 1 and 2), the following were significant: AC scores increased with age, with serum C-reactive protein, serum PO_4, and daily $CaCO_3$ dose. Serum intact PTH and serum albumin declined with higher AC scores. The number of patients with previous PTX was significantly higher in AC scores 3 and 4. Serum Al and peak serum Al after DFO were increased in AC scores 3 and 4. An inverse relationship ($P < 0.001$) was observed between declining serum PTH and rising peak serum Al after DFO. Serum $25(OH)D_3$ and the daily dose of vitamin D_3 were similar in all groups.

Associations between AC scores and bone parameters are reported in Table 2. The main results indicate that extensive AC (scores 3 and 4) are associated with lower bone activity characterized by significantly lower osteoclast resorption and fewer osteoclasts, and smaller osteoblast surface and double tetracycline-labeled surfaces with larger Al-stained surfaces. Bone histomorphometric parameters were not statistically different in patients with scores of 0 to 2. The increase of AC scores from score 0 to 2 was associated with higher serum PO_4 levels ($P<0.01$), older age ($P < 0.01$), and higher daily dose of $CaCO_3$ ($P < 0.001$) for patients with score 2.

For the entire population, significant colinearities were found between calcification score, age, PTH, peak Al after DFO, and bone variables, and, according to the multiple stepwise regression analysis, the AC score was positively associated with age ($P < 0.0001$), $CaCO_3$ dose ($P < 0.001$), and Al-stained surfaces ($P = 0.037$), and inversely associated with gender ($P = 0.012$) and osteoblast surface ($P < 0.001$).

Comparison of Patients with and without PTX

Twenty-three patients underwent subtotal PTX. Hyperparathyroidism recurred in six of them (PTH 398 to 975 pg/ml; five had AC scores 0 to 2, and one an AC score of 3). Distributions of serum PTH and some bone histomorphometry variables according to AC scores in the two populations are shown in Figure 1. For patients with and without PTX, the highest AC score was significantly associated with the lowest median serum PTH levels, and lower bone histomorphometric indexes, including (data not shown) osteoclast resorption surface ($P =$

Table 2. Calcium-phosphate metabolism and bone histomorphometry as a function of arterial calcification score in the overall ESRD population[a]

Variable	0 $n = 9$	1 $n = 10$	2 $n = 12$	3 $n = 11$	4 $n = 16$	KW (P value)	Trend (P value)	Group Differences
Serum PO_4 (mmol/L)	1.65 (1.48–2.10)	1.93 (1.57–2.84)	2.08 (1.51–2.68)	1.93 (1.34–2.80)	2.08 (1.06–3.15)	0.05	0.037	–
Serum Ca (mmol/L)	2.46 (2.0–2.65)	2.46 (2.11–2.65)	2.39 (2.30–2.69)	2.45 (2.25–2.56)	2.41 (2.18–2.54)	0.62	–	–
Serum PTH (pg/ml)	388 (38–758)	567 (175–1819)	316 (146–772)	202 (4–658)	71 (3–523)	0.0001	0.0001	4 *versus* 0–2
Serum Al (μmol/L)	0.70 (0.5–1.4)	0.60 (0.4–1.3)	1.20 (0.2–2.7)	1.9 (0.7–3.70)	1.6 (0.7–5.0)	0.003	0.001	3,4 *versus* 1
Peak Al after DFO (μmol/L)	1.8 (1.0–4.1)	1.45 (0.7–5.2)	3.0 (0.4–6.2)	4.6 (1.8–10.4)	5.15 (1.6–8.7)	0.0011	0.001	4 *versus* 0,1 3 *versus* 0–2
Serum $25(OH)D_3$ (nmol/L)	64 (32–103)	69 (24–177)	51 (13–609)	52 (9–135)	43 (5–76)	0.36	–	–
$CaCO_3$ (g Ca/d)	0.00 (0–1.20)	0.60 (0–1.50)	2.00 (0–3.60)	2.20 (1.20–3.60)	2.45 (1.2–4.80)	0.0001	0.0001	0,1 *versus* 2–4
$1,25(OH)_2D_3$ (μg/d)	0.10 (0–057)	0.15 (0–080)	0.25 (0–1.35)	0 (0–0.87)	0 (0–0.75)	0.65	–	–
Osteoclast Resorption (%)	3.4 (0.0–5.0)	2.31 (0.7–5.5)	1.81 (0–6.02)	1.73 (0.45–6.32)	0 (0.0–2.46)	0.001	0.0001	4 *versus* 0–2
Osteoclasts (n/mm^2)	1.4 (0.08–4.90)	1.88 (0.25–4.92)	1.62 (0.0–3.71)	1.12 (0.40–2.92)	0.12 (0.0–1.0)	0.001	0.002	4 *versus* 0–2
Osteoblast surface (%)	13.4 (0.42–29.8)	11.9 (3.67–18.3)	9.56 (0.0–16.33)	4.11 (0.30–16.84)	1.00 (0–4.9)	0.0001	0.0001	4 *versus* 0–2
Osteoid volume (%)	3.78 (3.14–17.43)	6.50 (2.54–9.33)	9.83 (1.0–20.0)	10.13 (1.40–22.18)	6.10 (0.20–54.0)	0.4679	–	–
Double labeled surfaces (%)	12.23 (0.0–17.4)	14.70 (3.03–28.8)	5.62 (0.0–26.8)	2.85 (0.0–24.3)	0.0 (0.0–2.40)	0.0001	0.0001	4 *versus* 0–2 3 *versus* 1
Al-stained surfaces (%)	0.0 (0.0–54.3)	0.0 (0.0–100)	8.95 (0.0–66.3)	39.0 (0.0–100)	78.0 (0.0–100)	0.004	0.001	3,4 *versus* 0–1

[a] PTH, parathormone; Al, aluminum; DFO, deferroxamine. Normal values: osteoid volume, 2 ± 1%; osteoblast surfaces, 4 ± 2%; osteoclast resorption surfaces, 0.4 ± 0.3%; osteoclasts, 0.15 ± 0.05/mm^2.

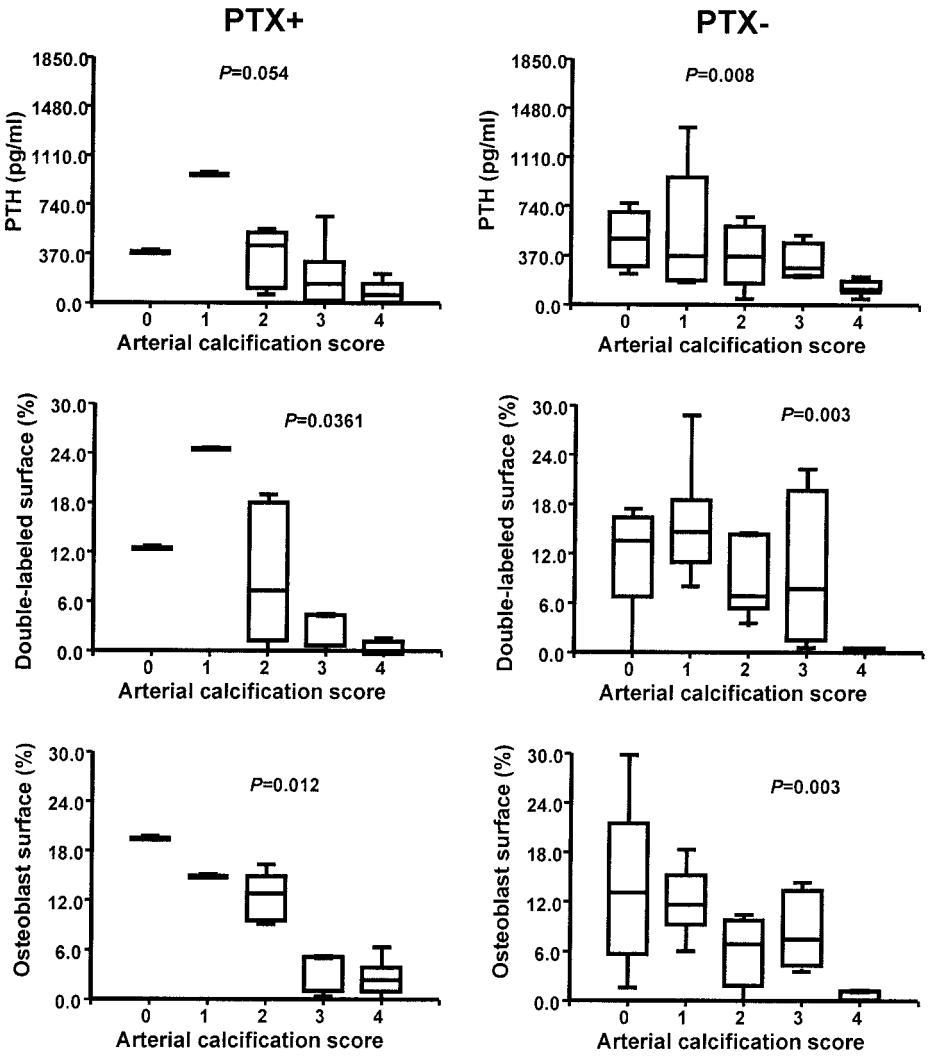

Figure 1. Serum parathyroid hormone (PTH), tetracycline double-labeled surface, and osteoblast surface as a function of arterial calcification (AC) scores, 0 to 4, in patients with parathyroidectomy (PTX+) or without (PTX−). The line in the middle of the box is the median. The top and bottom of the box are the 25th and 75th percentiles. The range is represented by T bars.

0.049 in PTX+ *versus* $P = 0.033$ in PTX−) and osteoclast numbers ($P = 0.028$ in PTX+ *versus* $P = 0.043$ in PTX−).

Comparison of Patients with Positive and Negative Al-Stained Bone Surfaces

Clinical and bone histomorphometry characteristics as a function of bone Al staining are shown in Table 3. Patients with positive Al bone staining (Bone Al+) had higher AC scores, were older, had been on HD for a longer time, and had lower serum albumin and higher serum cholesterol. These patients also had significantly higher serum Al, higher Al after DFO, higher daily $CaCO_3$ dose, and lower PTH. Patients with negative Al bone staining (Bone Al−) had significantly higher osteoclast resorption, and higher osteoblast and double tetracycline-labeled surfaces.

Distributions of serum PTH- and DFO-induced serum Al changes as a function of AC scores in Bone Al+ and Bone Al− groups are shown in Figure 2. Significant variations of PTH among the different AC score groups were observed for patients with Bone Al− (with significantly lower PTH associated with AC scores 3 and 4). In patients with Bone Al+, the PTH was significantly lower in patients with AC score 4. In patients with Bone Al+ DFO-induced serum, Al rose significantly in patients with AC scores of 3 and 4. In patients with Bone Al+, the percentage of Al−-stained surfaces did not significantly differ among the AC score groups ($P = 0.107$), but a positive trend ($P = 0.03$) was observed between AC scores 0 to 4. In patients with Bone Al−, the DFO-induced Al changes were comparable for all AC score groups. The distributions of serum bone histomorphometry variables according to AC scores as a function of Al staining are shown in Figure 3. In Bone Al+ or Bone Al− osteoclast number, osteoblast and double-labeled surfaces differed significantly according to AC scores, with lowest values for highest scores.

Discussion

The results of this study indicate that the extent of AC in patients with ESRD is associated with lower bone activity and adynamic bone disease. Decreased bone remodeling was significantly associated with aging and lower parathyroid "activity" in association with three factors: surgical PTX, Al overload, and high doses of Ca salts used as PO_4 binders. Hyperparathyroidism, as assessed by serum intact PTH levels

Table 3. Clinical, biochemical, and bone characteristics of patients as a function of positive (+) and negative (−) bone aluminum staining[a]

Variable	Bone Al+ (n = 33)	Bone Al− (n = 25)	P value
AC score	3 (0–4)	1 (0–4)	0.006
Age (yr)	58 (29–75)	48 (23–75)	0.043
Duration of HD (mo)	145 (12–304)	121 (12–204)	0.029
Smoking (pack-yr)	4.5 (0–32)	0 (0–51)	NS
Systolic BP (mmHg)	158 (89–193)	156 (99–199)	NS
Diastolic BP (mmHg)	84.5 (55–108)	82 (48–104)	NS
Total cholesterol (mmol/L)	5.55 (3.4–7.95)	4.75 (2.1–6.53)	0.011
Serum albumin (g/L)	38 (34–44)	39.3 (33–43)	0.023
Serum CRP (mg/L)	9 (1–30)	6.1 (0.9–41)	NS
PTX (yes/no)	16/31	7/22	NS
Serum Ca (mmol/L)	2.45 (2.22–2.69)	2.36 (2.0–2.60)	0.012
Serum PO_4 (mmol/L)	2.08 (1.45–315)	1.87 (1.06–2.84)	NS
Serum 25 $(OH)D_3$ (nmol/L)	51.5 (10–103)	43 (5–609)	NS
Serum Al (μmol/L)	1.75 (0.5–5.0)	0.90 (0.20–2.40)	0.0001
Peak serum Al after DFO (μmol/L)	5.15 (0.9–10.9)	1.8 (0.4–6.1)	0.0001
Δ Al after DFO (μmol/L)	3.2 (0.2–7.2)	0.95 (0.1–3.9)	0.0001
PTH (pg/ml)	158 (3–670)	316 (44–1838)	0.004
$CaCO_3$ (g elemental Ca/d)	2.4 (0–4.8)	1.75 (0–4.6)	0.007
1,25 $(OH)_2D_3$ (μg/d)	0 (0–0.87)	0.125 (0–1.35)	NS
Osteoclast resorption (%)	0.75 (0–6.02)	2.18 (0–6.32)	0.022
Osteoclasts (n/mm^2)	0.74 (0–3.71)	1.21 (0–4.90)	NS
Osteoblast surface (%)	3.70 (0–18.3)	9.12 (0–29.85)	0.047
Double-labeled surface (%)	1.5 (0–24.3)	9.00 (0–28.8)	0.035
Al-stained surface (%)	55.0 (5.4–100)	0 (0–0)	0.0001

[a] AC, arterial calcification; HD, hemodialysis; BP, blood pressure; CRP, C-reactive protein; PTX, parathyroidectomy; Al, aluminum; DFO, deferroxamine. Comparison by Wilcoxon test.

and bone histomorphometry, was inversely proportional to AC scores.

AC is an independent risk factor for death, predominantly from cardiovascular causes, for the general population and patients with ESRD on HD (4–6,10). The mechanisms responsible are complex, and abnormal mineral metabolism and PO_4 control have been considered major determinants. For many years, vascular and other soft tissue calcifications in patients with ESRD were considered to occur by passive mineral deposition, with elevated PO_4 and Ca-PO_4 product further increasing the supersaturation state that normally exists in plasma (8–10). The therapeutic interventions that affect total body Ca balance, like the use of high doses of Ca salts as a PO_4 binder and the administration of high doses of vitamin D, contribute to episodes of hypercalcemia, and aggravate the supersaturation state and contribute to the progression of AC (1–3,26). AC is now considered to be an actively regulated process influenced by tissue-specific cellular mechanisms (11–17), with other factors, such as age, inflammation, lipid metabolism, smoking, and genetic factors, also potentially involved (2,19,27–31).

An inverse relationship between AC and bone density has been documented in uremic patients (20). Disturbances of Ca and PO_4 metabolism in chronic kidney disease are associated with PTH secretion and uremic bone disease, but their role in the pathogenesis of AC remains uncertain. Although considered a cause of AC, the association of hyperparathyroidism and AC is not constant, and according to the most recent data, serum PTH and AC scores were not associated, and serum PTH was lower in patients with high AC scores (1,2,18,19). Arterial media calcifications were observed more frequently in patients with ESRD after PTX, and patients with the highest AC scores had the lowest PTH levels (19). Our data support the hypothesis that hyperparathyroidism, evaluated by bone histomorphometry and serum PTH determinations, does not play a direct role in AC pathogenesis. Bone histomorphometry indexes and serum PTH levels did not differ between patients with noncalcified arteries and those with AC scores of 1 and 2, but were significantly lower in patients with scores of 3 and 4 (Table 2). The univariate and multivariate regression analyses confirmed that bone activity, characterized by osteoblast surfaces and osteoclast numbers, was inversely related to the AC scores.

Recent investigation of diabetic LDL receptor–deficient mice showed that human PTH 1-34 (Teriparatide) inhibits vascular calcification and aortic osteogenic differentiation via a direct action and circulating osteopontin, and exerts a beneficial action on macrovascular disease (32). The differences among scores of 0 to 2 were not associated with significant

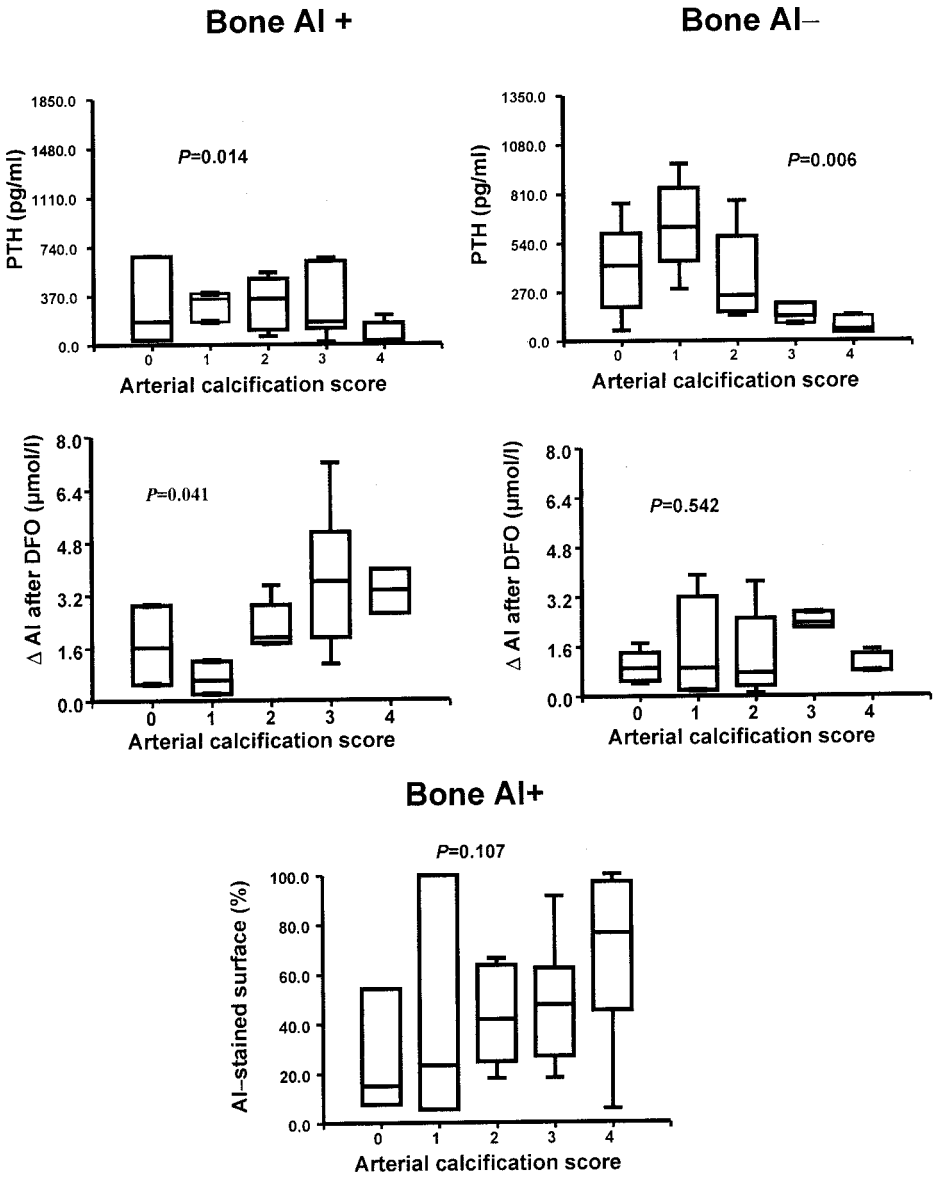

Figure 2. Serum parathyroid hormone (PTH)- and deferoxamine (DFO)-induced serum Al changes ((Al after DFO) as a function of arterial calcification (AC) scores, 0 to 4, for patients with positive (Bone Al+) or negative (Bone Al−) bone aluminum staining. Aluminum-stained surfaces and AC scores are provided for patients with Bone Al+ ($P = 0.03$ for trend).

differences in serum PTH or bone histomorphometry parameters, but were associated with increased serum PO_4 from score 0 to score 2. Although our data do not favor a direct action of PTH on AC score, elevated PO_4, by its direct influence on these two parameters could be the link associating hyperparathyroidism and AC. Serum PTH was significantly and inversely associated with the AC score and several bone histomorphometry indices, but our multivariate analysis did not retain an association with the AC score. Bone histomorphometry indices have higher sensitivity and reliability as markers of hyperparathyroidism than serum PTH. Serum intact PTH is not reliable as a sole indicator of bone turnover and also measures fragments, like 7 to 84 PTH, which antagonize some effects of the active whole hormone (33).

The second point is that patients with the highest AC scores had much lower tetracycline double-labeled surfaces, therefore suggesting that pathologically low bone remodeling (adynamic bone disease) was associated with extensive AC. Because a high percentage of patients with high AC scores had undergone PTX in the past, the possibility that these patient's high AC scores were related to previous hyperparathyroidism should be discussed. Nevertheless, as shown on Figure 1, the same relationships, *i.e.*, high AC scores and low serum PTH and lower bone activity, persisted in both PTX+ and PTX− patients. Moreover, hyperparathyroidism recurred in patients with lower AC scores.

Patients with high AC scores had higher baseline and serum Al after DFO and increased bone Al deposits. Although $Al(OH)_3$ prescriptions had been stopped for several years earlier, 33 patients had been on HD for many years, including when $Al(OH)_3$ was used as a PO_4 binder. According to our univariate analysis, baseline serum Al, DFO-induced serum Al changes, and Al-stained surfaces were inversely associated with all histomorphometric parameters of bone activity. The association of Al deposition with decreased bone remodeling could have several explanations. Bone Al deposition rises after

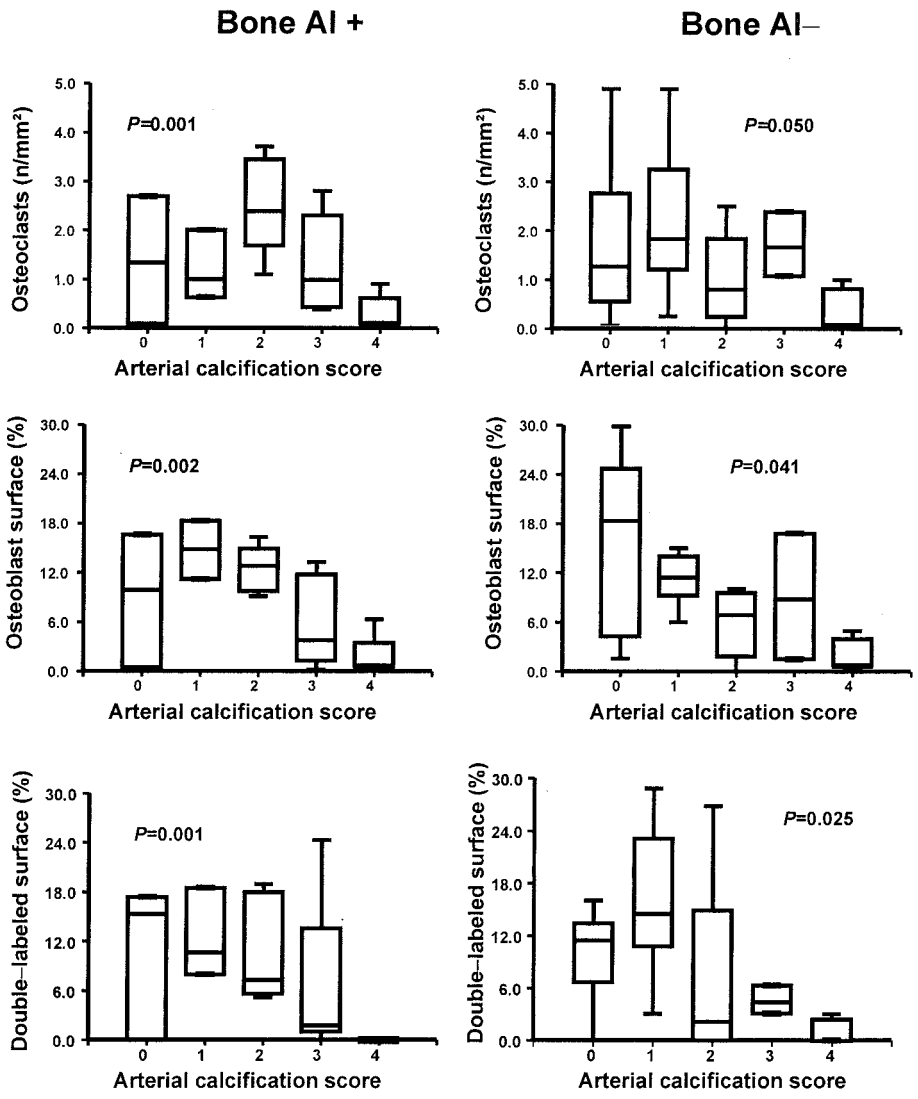

Figure 3. Osteoclast number, osteoblast surface, and tetracycline double-labeled surfaces as a function of arterial calcification (AC) scores, 0 to 4, in patients with positive (Bone Al+) and negative (Bone Al−) bone aluminum staining.

subtotal PTX, and it is likely that low PTH and low remodeling could facilitate bone Al deposition (34). On the other hand, a number of clinical and experimental studies demonstrated that Al reduces circulating PTH by decreasing its synthesis and release (35,36). As shown on Figures 2 and 3, the inverse relationships between histomorphometry indexes of bone activity and serum PTH were observed, regardless of whether patients had bone Al deposits or not, and, for those patients with these deposits, the various indexes of Al overload did not differ statistically among the different AC scores, but a trend ($P = 0.03$) was observed between AC scores and Al-stained surfaces. This finding would seem to suggest that the association between Al overload and AC is indirect, reflecting PTH suppression. Nevertheless, multivariate analysis of parameters for our entire ESRD population indicates that the extent of bone Al deposits is an independent factor associated with AC. This "direct" effect could be mediated by significantly higher serum Ca (Table 3) and hypercalcemic episodes (also suppressing PTH) frequently observed in patients with Al overload (37).

A few studies have shown that low serum PTH and hypoparathyroidism are predictors of mortality of patients with ESRD (38–40). Several mechanisms have been implicated (including aging, which is associated with more AC in parallel with higher frequency of adynamic bone). One explanation could be that lower serum PTH and adynamic bone disease are associated with malnutrition and inflammation, which are highly prevalent in patients with ESRD (38,39). Lower serum albumin and higher serum C-reactive protein were observed in our patients with higher AC scores and adynamic bone, but were not directly associated with bone parameters. The results of this study indicate that lower PTH is associated with AC, which is an independent risk factor for cardiovascular and all-cause mortality, and could explain the association between hypoparathyroidism and mortality.

This study has several limitations. The first is the semiquantitative AC score, the sensitivity of which is relatively low. The second is the observational nature of the study and the fact that the study concerns prevalent patients, thereby making difficult the reconstitution of the natural history of bone disease and its

association with AC. To analyze more precisely the natural history of AC, and specifically the links between mineral metabolism and/or bone turnover and vascular calcifications, interventional studies with long-term follow-up would be necessary.

In conclusion, high AC scores were associated with bone histomorphometry values suggestive of low bone activity and adynamic bone disease. This observation suggests that therapeutic interventions associated with excessive decrease of parathyroid activity (PTX, Al load) favor lower bone turnover and adynamic bone disease that, in combination with interventions that increase the Ca balance, could influence the development and progression of AC. Hyperparathyroidism does not seem to contribute directly to the pathogenesis of AC and an inverse relationship was observed between the serum PTH level and bone histomorphometry indices. The association between moderate AC and parathyroid activity could result from the direct action of hyperphosphatemia on AC and parathyroid activity.

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