Ankle-Brachial Index and Bone Turnover in Patients on Dialysis

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ABSTRACT

An association between atherosclerosis and osteoporosis has been reported in several studies. This association could result from local intrasosseous atherosclerosis and ischemia, which is shown by limb osteoporosis in patients with peripheral artery disease (PAD), but also could result from bidirectional communication between the skeleton and blood vessels. Systemic bone disorders and PAD are frequent in ESRD. Here, we investigated the possible interaction of these disorders. For 65 prevalent nondiabetic patients on hemodialysis, we measured ankle-brachial pressure index (ABix) and evaluated mineral and bone disorders with bone histomorphometry. In prevalent patients on hemodialysis, PAD (ABix ≤ 0.9 or > 1.4/incompressible) was associated with low bone turnover and pronounced osteoblast resistance to parathyroid hormone (PTH), which is indicated by decreased double-labeled surface and osteoblast surface (P<0.001). Higher osteoblast resistance to PTH in patients with PAD was characterized by weaker correlation coefficients (slopes) between serum PTH and double-labeled surface (P=0.02) or osteoblast surface (P=0.03). The correlations between osteoclast number or eroded surface and serum mineral parameters, including PTH, did not differ for subjects with normal ABix and PAD. Common vascular risk factors (dyslipidemia, smoking, and sex) were similar for normal, low, and incompressible ABix. Patients with PAD were older and had high C-reactive protein levels and longer hemodialysis vintage. These results indicate that, in prevalent nondiabetic patients with ESRD, PAD associates with low bone turnover and pronounced osteoblast resistance to PTH.


The bone–vascular axis concept emerged from clinical and experimental findings linking bone and arterial remodeling and changes. Observations in diverse populations have revealed associations between atherosclerosis and vascular calcifications with decreased bone mineral density (BMD), risk for vertebral or hip fracture, low bone turnover, and cardiovascular disease. The biologic link between vascular disease and bone changes is certainly part of the aging process, but in many studies, these bone–vessel associations remained significant after adjustment for age, suggesting an age-independent causal relationship. Nevertheless, the factors or mechanisms underlying these associations are not well understood and could result from common mechanisms acting on both systems (e.g., inflammation, hyperlipidemia, diabetes, smoking, or generalized arterial disease per se). Blood vessels play a major role in osteogenesis, and factors affecting the blood supply to bones could have major effects on bone remodeling and structure.
Ischemia resulting from intraosseous atherosclerosis and arterial calcifications could explain the association between osteoporosis and decreased BMD. The link between compromised bone circulation and osteoporosis was documented by the observation linking lower limb bone mineral content and abnormal bone turnover with increased risk of fractures in men with ischemic atherosclerotic peripheral artery disease (PAD).16–19

Lower limb PAD is a frequent complication of ESRD, and low ankle-brachial pressure index (ABix) or incompressible arteries are associated with all-cause and cardiovascular mortality.20–23 In general populations, associations between PAD and BMD are observed at the local (lower limb) level and frequently, in the absence of overt mineral metabolism disorders; in patients with CKD and ESRD, those interactions coexist frequently with mineral and bone disorders, including perturbed parathyroid hormone (PTH) and vitamin D metabolisms. In this study, we had the opportunity to analyze the relationships between ABix-assessed subclinical PAD and mineral and bone abnormalities, including systemic bone, on the basis of histomorphometry of iliac crest biopsies. These results indicate that, in prevalent nondiabetic patients with ESRD, PAD was associated with low bone turnover and pronounced osteoblast resistance to PTH.

RESULTS

Patient Characteristics

We studied 65 patients whose clinical characteristics, brachial and lower limb systolic BP (SBP), and ABix are summarized in Table 1. ABix was normal in 30 of 65 (46.2%) patients, ABix and lower limb SBP, and ABix are summarized in Table 1. ABix was normal in 30 of 65 (46.2%) patients, ABix and lower limb SBP, and ABix are summarized in Table 1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal (n=30)</th>
<th>Low (n=11)</th>
<th>&gt;1.4 (n=24)</th>
<th>ANOVA P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>44 (29 to 50)</td>
<td>59* (52 to 65)</td>
<td>59* (52 to 64)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.6 (21.3 to 24.1)</td>
<td>20.9 (17.1 to 27.9)</td>
<td>22.5 (20.3 to 23.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Vintage (mo)</td>
<td>48 (29 to 65)</td>
<td>85* (36 to 104)</td>
<td>144* (96 to 173)</td>
<td>0.001</td>
</tr>
<tr>
<td>Smoking (pack/yr)</td>
<td>0 (0 to 3)</td>
<td>0 (0 to 25)</td>
<td>5.5 (0 to 16)</td>
<td>NS</td>
</tr>
<tr>
<td>PTX (no.)</td>
<td>4/30 (13.3%)</td>
<td>5/11* (45.5%)</td>
<td>13/24* (54.2%)</td>
<td>0.004</td>
</tr>
<tr>
<td>CaCO₃ (g/d)</td>
<td>1.2 (0.8 to 1.2)</td>
<td>1.8 (1.4 to 2.4)</td>
<td>2.4* (2 to 2.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Brachial SBP (mmHg)</td>
<td>147 (134 to 164)</td>
<td>147 (131 to 156)</td>
<td>156 (138 to 168)</td>
<td>NS</td>
</tr>
<tr>
<td>Lower limb SBP (mmHg)</td>
<td>175 (164 to 185)</td>
<td>126* (104 to 139)</td>
<td>300* (250 to 300)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ABix</td>
<td>1.14 (1.10 to 1.16)</td>
<td>0.86* (0.65 to 0.89)</td>
<td>1.76* (1.52 to 1.9)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are medians (95% confidence intervals). Kruskal–Wallis one-way ANOVA on ranks.

aSignificantly different from normal ABix.
bSignificantly different from low ABix and ABix>1.4.

Table 1. Clinical characteristics of patients with ESRD

Blood chemistries are reported in Table 2 according to ABix. Patients with PAD had significantly higher serum high-sensitivity C-reactive protein (hCRP) and lower serum albumin. All others biochemical parameters, including PTH, were not significantly different among the three groups.

Bone Histomorphometry

Bone histomorphometry parameters are given in Table 3. Osteoblast surface per bone surface (Ob.S/BS) and double-labeled surface (dLS/BS) were significantly lower in patients with PAD. Patients with PAD had higher aluminum-stained surface but similar bone volume and osteoid volume. In multiple regression analyses, dLS/BS was positively associated with serum PTH (P<0.001), and it was negatively associated with serum hCRP (P<0.001) but not negatively associated with aluminum-stained surfaces (P=0.27). As shown in Figure 1 in pooled abnormal ABix, after adjustment for age and serum hCRP, the correlations between serum PTH, dLS/BS, and Ob.S/BS were positive for normal and abnormal ABix (Figure 1). Upward shifts with significantly steeper slopes (β-coefficient) of the correlation between dLS/BS and PTH (mean β-coefficient±SD; 21±24.4 versus 5.12±30.3; T=2.34; P=0.03) and between Ob.S/BS and PTH (mean β-coefficient±SD; 17±18.6 versus 8.4±10.2; T=2.25; P=0.03) were observed for patients with normal ABix. Osteocalcin number/per millimeter² (N.Oc/T.A) and eroded surface (ES/BS) did not differ among the three groups (Figure 1). The correlations between circulating PTH, N.Oc/T.A, and ES/BS, were positive and similar for normal and abnormal ABix. Significant negative serum PTH–adjusted correlations were observed between serum hCRP, Ob.S/BS, and dLS/BS (Figure 2).

Corrected univariate correlations showed that PAD was positively associated with serum CRP, age, dialysis vintage, and calcium carbonate dose and inversely related to dLS/BS, Ob.S/BS, aluminum-stained surfaces, and PTX (Table 4). Because of multiple colinearities (for example, correlation between vintage and PTX: R=0.3615; P=0.004 or correlation between
vintage and aluminum-stained surface: R = 0.5365; P < 0.001), multivariate stepwise correlation analysis identified only hCRP, vintage, and dLS/BS as significant cofactors associated with PAD (Table 5).

DISCUSSION

Our results indicated that, in patients with prevalent hemodialysis, subclinical PAD was associated with low bone turnover and more pronounced osteoblast resistance to PTH, which was assessed by decreased dLS/BS and Ob.S/BS and lower correlation \( \beta \)-coefficients (slopes) between serum PTH and dLS/BS or Ob.S/BS (Figure 1, A and B). The correlations between PAD and N.Oc/T.A or ES/BS did not differ from subjects with normal ABix. These bone–artery associations remained significant after adjustment for age, serum hCRP, and hemodialysis vintage. Although hemodialysis vintage and serum hCRP were independently associated with PAD, serum mineral parameters, including PTH, did not differ significantly between groups. Unlike the published association between the calcium phosphate binders doses and aortic calcifications,8 in multivariate analyses, this association was not observed in peripheral arteries with abnormal ABix.

Decreased femoral neck or total hip BMD and increased risk of fractures were reported to be associated with PAD,16,18,19 and bone perfusion was reduced in osteoporotic patients compared with normal and osteopenic subjects.15 In a study on patients with unilateral PAD, Laroche et al.16 reported that BMD was significantly lower in the affected limb compared with the unaffected limb. Unlike the reported association between PAD and local femoral BMD, our results indicated an association between PAD and systemic bone changes in non-weight-bearing anterior iliac crest.

Our study population did not include patients with diabetes, and the only common bone–artery risk factor was higher hCRP in patients with abnormal ABix (Tables 2 and 5). In an experimental in vivo study, Hjortnaes et al.24 showed
the role of inflammation in the inverse relationship between osteoporotic bone remodeling and arterial and aortic valve calcifications. Inflammation is associated with atherosclerosis, a strong inductor of vascular calcifications, and a modulator of osteoblast activity. Independent of serum PTH, high serum hCRP was positively associated with abnormal ABix and inversely correlated with dLS/BS and Ob.S/BS (Figure 2).

However, the correlations between PTH and bone turnover markers were independent (Figure 1) of hCRP, suggesting that the resistance to PTH could be associated with arterial changes. As shown in Figure 1 and Table 5, an abnormal ABix was associated with low bone turnover, principally affecting osteoblasts. In contrast, N.Oc/T.A and resorption activity did not differ significantly between patient groups. In a study on the general population, Pennisi et al. showed that peripheral vessels atherosclerosis was associated with biochemistry parameters suggestive of reduced bone formation and abnormal bone turnover. PTH resistance in patients with ESRD is well known and multifactorial, including abnormal PTH signaling. Downregulation of PTH/PTH-related protein-receptor type I (PTHrP1) gene expression and mRNA in osteoblasts, presence of antagonistic C-terminal PTH fragments, hyperlipidemia-induced oxidant stress, uremic milieu, uremic toxins, inflammation, and aging are all mechanisms accounting for skeletal resistance to PTH and adynamic bone.

Could defective PTH signaling play a role in the development of bone–vascular axis abnormalities? Arterial calcifications are highly prevalent in subjects with low bone turnover and abnormal ABix. Experimental data showed that, by signaling through the PTH/PTHrP receptor (PTH1R), parathyroid PTH1–34 inhibited bovine vascular smooth muscle cell (VSMC) calcification and blocked the calcifying action of calcitriol. Shao et al. showed that teriparatide (human PTH1–34) inhibited osteogenic vascular calcification in diabetic LDL receptor-deficient mice. Cheng et al. showed that activation of VSMC PTH1R inhibited the Wnt/β-Catenin signaling pathway and prevented aortic calcifications and fibrosis in diabetic arteriosclerosis. The role of PTH1R signaling through PTH or PTHrP in arterial remodeling remains the subject of investigations, but experimental data indicate that PTHrP inhibited VSMC migration and proliferation and neointimal formation. PTH1R signaling plays an important role in bone health and vascular calcification.

Figure 1. Correlations between serum PTH and bone histomorphometry parameters. (A) Ob.S/BS, (B) dLS/BS, (C) N.Oc/T.A, and (D) ES/BS for patients with normal ABix (white circles) and patients with abnormal ABix (black triangles). The correlations are adjusted to serum CRP.

role in the skeletal response to PTH through the control of osteocyte activation of SOST/sclerostin. In the presence of PTH resistance and impaired signaling, the transcriptional suppression of sclerostin production might be impaired.\textsuperscript{45,46} Resulting from experimental data, these hypotheses were not tested in this population, but serum sclerostin is increased in patients with ESRD\textsuperscript{47,48} and associated with cardiovascular calcifications.\textsuperscript{49} Our findings support, in a human ESRD population, the common role of inflammation in bone and cardiovascular remodeling, which was shown experimentally by Hjortnaes \textit{et al.},\textsuperscript{24} but the association between low bone turnover and arterial remodeling is not new.\textsuperscript{8,50} Hjortnaes \textit{et al.}\textsuperscript{24} confirm the dissociation between serum PTH concentrations and bone histology and PTH concentration with clinical disease. Herein, the presence of subclinical PAD was not associated with any differences in serum PTH but was associated with a different clinical effect, which was characterized by skeletal resistance to PTH involving, more importantly, osteoblast function and number compared with osteoclast and bone erosion (Figure 3). Bone remodeling is maintained by a balance between bone erosion and bone formation and dependent on bidirectional communication between osteoblasts and osteoclasts through paracrine factors (receptor activator of NF-\kappaB [RANK] ligand, osteoprotegerin, and ephrinB2 ligand), cell–cell contact, and cell–bone matrix interactions.\textsuperscript{50} Lesser coupling between bone erosion and bone

Table 4. Univariate correlations between PAD status (0, normal; 1, PAD) and clinical and biochemistry parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>( R ) Value</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>0.5936</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vintage (mo)</td>
<td>0.4878</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CaCO\textsubscript{3} (g/d)</td>
<td>0.5230</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hCRP (log mg/L)</td>
<td>0.7512</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>-0.5920</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>dLS/BS (%)</td>
<td>-0.5627</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ob.S/BS (%)</td>
<td>-0.5054</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N.Oc/T.A (mm\textsuperscript{2})</td>
<td>-0.3855</td>
<td>0.002</td>
</tr>
<tr>
<td>Aluminum-stained surface (%)</td>
<td>0.3539</td>
<td>0.004</td>
</tr>
<tr>
<td>PTX (no, 0; yes, 1)</td>
<td>0.3570</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Figure 2. Correlations between serum hCRP and bone histomorphometry parameters. (A) Ob.S/BS, (B) dLS/BS, (C) N.Oc/T.A, and (D) ES/BS in patients with normal ABix (white circles) and patients with abnormal ABix (black triangles). The correlations are adjusted to serum PTH.
downregulation of PTH/PTHrP and activation of osteoclasts and IL-1, could cause an imbalance in bone remodeling by dLS/BS (%)

Log hCRP (mg/L) 6.087

Age (yr) 0.850

CaCO₃ (g/d) 1.193

N.Oc/T.A (mm²) 0.696

PTX (no, 0; yes, 1) 0.597

Aluminum-stained surfaces (%) 0.050

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T Value</th>
<th>P Value</th>
<th>Partial R² Adjusted for Rest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vintage (mo)</td>
<td>4.040</td>
<td>&lt;0.001</td>
<td>0.2111</td>
</tr>
<tr>
<td>dLS/BS (%)</td>
<td>2.877</td>
<td>&lt;0.01</td>
<td>0.1195</td>
</tr>
<tr>
<td>Log hCRP (mg/L)</td>
<td>6.087</td>
<td>&lt;0.001</td>
<td>0.3669</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>0.850</td>
<td>0.40</td>
<td>NA</td>
</tr>
<tr>
<td>CaCO₃ (g/d)</td>
<td>1.193</td>
<td>0.24</td>
<td>NA</td>
</tr>
<tr>
<td>N.Oc/T.A (mm²)</td>
<td>0.696</td>
<td>0.49</td>
<td>NA</td>
</tr>
<tr>
<td>PTX (no, 0; yes, 1)</td>
<td>0.597</td>
<td>0.55</td>
<td>NA</td>
</tr>
<tr>
<td>Aluminum-stained surfaces (%)</td>
<td>0.0509</td>
<td>0.96</td>
<td>NA</td>
</tr>
</tbody>
</table>

R²=0.6843.

**Table 5. Stepwise regression report for PAD status (0, normal; 1, PAD)**

**Figure 3.** Correlation between bone histomorphometry parameters. dLS/BS and ES/BS in patients with normal ABix (white circles) and patients with abnormal ABix (black triangles).

formation with decreased number and activity of osteoblasts was observed in several conditions, such as skeletal unloading during prolonged bed rest, aluminum-related osteodystrophy in patients on dialysis, and chronic inflammation. In this study, the PTH- and CRP-adjusted correlation between aluminum-stained surfaces and dLS/BS was not significant, but patients with PAD had microinflammation and increased serum CRP. We can speculate that the microinflammation could play a role in bone erosion uncoupling. As shown on Figure 2, serum CRP was inversely correlated with dLS/BS and Ob.S/BS but less correlated or not correlated with N.Oc/T.A and ES/BS. Inflammation and proinflammatory cytokines, such as TNF-α and IL-1, could cause an imbalance in bone remodeling by downregulation of PTH/PTHrP and activation of osteoclasts through classic or alternative RANK/RANK ligand pathways favoring bone erosion.

The possible role of abnormal PTH signaling in arterial remodeling and vascular pathology remains hypothetical, because observational studies on the basis of correlations studies and associations are not proof of causality. This limitation is an important limitation of this study. Another limitation concerns the limited number of patients and clinical characteristics of the population, which included relatively young subjects with primary kidney disease, excluding diabetes, treated in the 1990s. This population differs markedly from the population treated today, which includes higher percentages of patients with diabetes and older patients with overt cardiovascular comorbidities and shorter vintage. The treatment modalities have evolved with withdrawal of aluminum-containing phosphate binders and the use of noncalcium-containing phosphate binders, the wider use of vitamin D receptor agonists and cholecalciferol supplementation, the prescription of calcimimetics, and the improvement of hemodialysis techniques.

In summary, our results showed that, in prevalent non-diabetic patients with ESRD, the PAD was associated with low bone turnover and low bone formation with pronounced osteoblast resistance to PTH.

**CONCISE METHODS**

**Patients**

With the exception of 1 patient, the population of 65 prevalent non-diabetic patients on hemodialysis was previously detailed. Inclusion criteria were (1) hemodialysis vintage ≥12 months and (2) absence of clinical history of cardiovascular disease, including history of lower limb arteries angioplasty, stenting, amputation, or trophic disorders (ulcerations or gangrene). Dialysis duration was individually tailored (4–6 hours three times per week) to control body fluids and blood chemistries. The bicarbonate dialysate was prepared using double reverse osmosis-treated water with 1.5 or 1.75 mmol/L Ca according to the serum Ca-Pt equilibrium and the need for active vitamin D₃ (1α-OH-D₃ prescribed in eight patients). Although CaCO₃ was used exclusively as a PO₄ binder at the time of the study, 28 patients had taken aluminum hydroxide in the past; 12 patients underwent subtotal PTX, and 10 patients underwent total PTX with heterotopic autotransplantation into the forearm. PTX had been performed 20–70 months before the study. Erythropoietin was administered to maintain hemoglobin ≥100 g/L when necessary. All subjects gave informed written consent to participate in the study, which was approved by our Institutional Review Board in accordance with the Declaration of Helsinki.

**ABix**

Lower limb artery status was on the basis of ABix measurement and the ratio between SBP in the ankle (arteria tibialis posterior or dorsalis pedis) and the arm (arteria brachialis). SBPs were measured in a supine position after 15 minutes of rest. Brachial artery SBP was measured twice in the arm without arteriovenous shunt and averaged. Ankle SBP was measured twice in each foot and averaged. SBPs were measured with a Doppler SEGA M842 8-MHz Unit (Société Électro- technique Générale et Appliquée, Paris, France) during cuff deflation. Patients were divided in three groups according to their ABix values: normal ABix, 0.9–1.3; low ABix, <0.9; >1.4 (i.e., incompressible).
Bone Histomorphometry
Diagnostic anterior iliac crest bone biopsies were obtained after dLS/BS according to the schedule of 2 days on tetracycline, 10 days off tetracycline, and 2 days on tetracycline as previously detailed. The extent of the dLS/BS (percent) was measured on two unstained 10-μm-thick sections. On three Toluidine blue-stained 5-μm-thick sections, bone volume (percent), Ob.S/BS (percent), ES/BS (percent), and N.Oc/T.Ar were assessed. Bone Al staining was expressed as the percentage of the trabecular surface stained. All measurements were made using an eyepiece reticle (Carl Zeiss Integral Plate II, Oberkochen, Germany).

Blood Chemistries
Blood chemistries, including serum Ca and P, blood lipids, blood glucose, uric acid, and serum albumin, are the mean values of all measurements in the 6 months preceding the ABix measurements. Routine biochemical parameters were determined by standard methods using autoanalyzers. Blood samples for measurement of bone alkaline phosphatase serum levels (Quidel MicroVue BAP EIA), 25 hydroxyvitamin D, and intact PTH (N-IRMA; Nichols Institute) were determined every 3 months, and the reported values are the average of two measurements done during the 6 months preceding the bone biopsy. Bone biopsy and measurement of ABix were done the same month.

Statistical Analyses
Data are expressed as medians and 95% confidence intervals. The primary analysis concerned ABix subgroup normal, low, or >1.4 comparison with Kruskal–Wallis one-way ANOVA on ranks and Bonferroni multiple comparison Z-test value. For the univariate (Pearson or Spearman correlation coefficient), and multivariate regression studies, low and incompressible ABix groups were pooled and considered to have PAD. The correlations slopes are expressed as β-coefficients±SD and compared by t test. For these correlations, artery disease status was used as a dummy variable (0, normal ABix; 1, PAD). Multiple regression analyses were performed using the subset of univariate analysis-selected independent variables (after Bonferroni correction for the number of correlations studied). All analyses were calculated using NCSS 2000 (Gerry Hintze, Kaysville, UT).

ACKNOWLEDGMENTS
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DISCLOSURES
None.

REFERENCES


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