Serum Aluminum Levels as a Reflection of Renal Osteodystrophy Status and Bone Surface Aluminum Staining 1

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ABSTRACT
Twenty eight (14%) out of 196 patients in a regional dialysis population were found to have serum aluminum levels ≥5 μmol/L or ≥135 μg/L; 21 consented to undergo a bone biopsy to identify the spectrum of renal osteodystrophy associated with this degree of hyperaluminemia. Both the Aluminon reagent and the acid solochrome azurine (ASA) stain were used to identify aluminum deposits. A control group of 13 patients with biochemical and histological evidence of severe secondary hyperparathyroidism was used to contrast the measured parameters of bone histology in the hyperaluminemic group. Al(OH)3 was used as the principal phosphate binder in all patients. In the hyperaluminemic group, 67% had either dialysis osteomalacia or aplastic bone lesions, and all except one aplastic lesion were positive for bone surface aluminum deposits by the Aluminon stain. The Aluminon stain was also positive in one of three cases of osteitis fibrosa and three of four mild lesions, whereas it was negative in all biopsies from the control group. However, the ASA stain was positive in all biopsies from the hyperaluminemic group and in 11 of 13 control biopsies from the patients with "pure" osteitis fibrosa. For all biopsy data from both groups, there were significant (P < 0.01) negative correlations between the ASA-stained surface aluminum deposits and resorption indices (total eroded surface, r = −0.68; surface osteoclast counts, r = −0.53) and indices of bone formation (surface osteoblast counts, r = −0.61; mineral apposition rate, r = −0.63; bone formation rate, r = −0.69). These correlations were not significant for Aluminon-stained surface deposits with the exception of the bone formation indices, which had lower correlation coefficients (r = −0.44). These data suggest that hyperaluminemia ≥5 μmol/L has a predictive value to identify impaired mineralization in dialysis patients that is high enough to affect clinical decision making. However, the more sensitive ASA stain identifies surface aluminum across the whole spectrum of renal osteodystrophy and is consistent with a toxic role for aluminum at any level of exposure.

Key Words: Serum aluminum, bone aluminum stains, renal osteodystrophy, chronic renal failure, hemodialysis

There is a strong association linking aluminum accumulation in renal dialysis patients, with both dementia (1,2) and severe osteomalacia (2–5). Aluminum can be identified by two histochemical stains in bone biopsy sections; the application of the first stain, which uses aurin tricarboxylic acid (the "Aluminon" stain) has been used extensively as an aid to classifying renal osteodystrophy, as well as a diagnostic test for clinical aluminum toxicity. Thus, linear aluminum deposits can usually be identified at the bone-osteoid interface in biopsies obtained from patients with both the dialysis osteomalacia syndrome and its variant, "aplastic" or "adynamic" bone disease (6–10). Both of these histological subtypes of renal osteodystrophy are characterized by severely impaired mineralization as assessed by the failure of the osteoid seams to take up tetracycline labels, whereas the location of aluminum deposits at the mineralization site has lent additional weight to its pathogenetic role in these conditions. In contrast, other forms of renal osteodystrophy, in particular those associated with histological evidence of hyperparathyroidism, stain positively with the Aluminon reagent only infrequently (7,9,10). A second staining technique for aluminum with acid solochrome azurine (ASA) has recently been applied to bone biopsies from a limited group of patients with renal osteodystrophy; by this technique, the deposition of aluminum in bone appears to be seen in a much wider spectrum of histological lesions than that identified by the Aluminon reagent (11).

Although there is a general consensus among ne-
The use of aluminum-based phosphate-binding gels should be restricted as much as possible to prevent the emergence of aluminum toxicity in both brain and bone (12,13), the alternate phosphate-binding drug, calcium carbonate, is not always successful in controlling serum phosphate levels (13). Indeed, the use of calcium carbonate is frequently restricted by the occurrence of hypercalcemia in dialysis patients, particularly when it is necessary to use vitamin D metabolites concurrently to control secondary hyperparathyroidism. How can the physician monitor the risk of developing aluminum toxicity? Smith et al. have suggested that bone biopsies are the only means by which to correctly identify patients at risk (9). However, bone biopsies are invasive, and moreover, the techniques for processing bone histology are not widely available. Serum aluminum levels are more easily obtained and the increase in serum aluminum can be measured after a single challenge with the chelating agent, desferrioxamine (DFO), by using the DFO infusion test, which has been advocated as a reliable way to identify patients with aluminum-associated dialysis osteomalacia (14,15). However, Hodsman et al. subsequently reported that the DFO infusion test was no more sensitive than the baseline serum aluminum in distinguishing dialysis osteomalacia from secondary hyperparathyroidism, by using a diagnostic cutoff in serum aluminum of 5 μmol/L, equal to 1 SD, above the reference dialysis population mean (16). At least three additional reports have also concluded that an empirical cutoff value for serum aluminum of around 5 μmol/L is predictive for bone histological evidence of aluminum toxicity (17–19).

The purpose of this article is twofold. First, we wished to explore further the predictive value of serum aluminum levels in excess of 5 μmol/L to identify patients with bone aluminum toxicity. To do this, we systematically evaluated the bone biopsies from patients whose aluminum levels exceeded this value at our annual screening of the Southwestern Ontario regional dialysis population. Second, we sought to explore further the behavior of the ASA stain in this group of dialysis patients at “high-risk” for aluminum toxicity and to thereby determine whether this additional stain provides any further information on the mechanism of aluminum toxicity in bone. As a control group, we have selected patients who had unequivocal evidence of severe secondary hyperparathyroidism because this group of patients is generally thought of as being “protected” from aluminum toxicity (20).

PATIENT POPULATION

Group 1—Hyperaluminemia

Out of a regional hemodialysis population of 196 patients, 28 (14%) were found to have hyperaluminemia, defined as a serum aluminum level ≥5.0 μmol/L (1 SD above the population mean). In this population, Al(OH)3 was used as the primary agent for controlling hyperphosphatemia. Of these 28 patients, 21 consented to undergo a transiliac bone biopsy to survey the spectrum of the renal osteodystrophy associated with this degree of hyperaluminemia; they form the basis of this report.

Group 2—Osteitis Fibrosa

The clinical, biochemical, and histologic findings in Group 1 are contrasted with those of 13 hemodialysis patients presenting over a 5-yr period with unequivocal evidence of severe secondary hyperparathyroidism. These patients had at least two out of three biochemical markers for osteitis fibrosa, 10 had hypercalcemia, 12 had serum immunoreactive parathyroid hormone (i-PTH) levels more than 10 times the normal upper limit of the assay (see below), and 12 had serum alkaline phosphatase levels greater than 150 IU/L; all 13 patients had bone biopsies to confirm the histological evidence of osteitis fibrosa including: (1) peritrabecular fibrosis ≥0.5% total tissue volume; (2) markedly increased bone erosion surfaces (>10% total cancellous surface); and (3) no evidence of osteomalacia as evidenced by normal bone formation rates calculated from the tetracycline-labeled surfaces. All patients were being treated with Al(OH)3 as the primary oral phosphate binder at the time of diagnosis.

METHODS

Serum calcium (Ca), inorganic phosphate (P), and alkaline phosphatase were measured by standard automated techniques. Serum i-PTH levels were measured by the Incstar midmolecule assay (reference range, 5 to 85 pmol/L; Incstar Corp., Stillwater, MN). The correlations described in this report between serum i-PTH levels by this assay and bone histological parameters of bone resorption and formation are at least as significant as those found by Andress et al., who used an “intact” PTH measurement by RIA (21). All serum aluminum levels were measured by flameless atomic absorption spectrophotometry in a central regional laboratory as previously described (22). Hyperaluminemia (serum aluminum ≥5.0 μmol/L or 135 μg/L) was identified at the routine biannual screening performed in January 1986, and patients whose values were elevated to this threshold were labeled hyperaluminemic if the serum level was similarly elevated 6 months earlier or if it was confirmed by a repeated measurement. The intraindividual coefficient of variation for serum aluminum levels measured weekly over 6 weeks was found to be ±12% (16). Transiliac bone biopsies were obtained with a 6- or 8-mm Bordier trephine after in...
utio tetracycline labeling and were processed for histomorphometry as previously described (23); tetracycline labels were given orally or by intravenous infusion, 14 days apart. Cancellous surface staining for linear aluminum deposits was identified by two techniques: the Aluminon stain with aurin tricarboxyclic acid, modified from the study by Buchanan et al. (24) and the ASA stain as recently described by Kaye et al. (11). Quantitative measurements were made of the following histological parameters: total bone volume (percent BV/TV), osteoid volume (percent OV/BV), osteoid seam thickness (0 Th, micrometers), total eroded cancellous surface (percent ES/BS), total cancellous surface positively stained for aluminum by the Aluminon method (percent AI/BS), and total cancellous surface positively stained by the ASA method (percent ASA/BS). We have represented active cellular surfaces by direct counts of osteoblasts and osteoclasts per unit length of cancellous surface (NOb/cm and NOc/cm, perimeter, respectively). Peritrabecular fibrosis was expressed per total tissue volume (percent fibrosis/TV). The mineral apposition rates (MAR, micrometers per day) were calculated from the double labels. The bone formation rates (surface referent, BFR; cubic micrometers per square micrometer per year) were calculated using the tetracycline data, from the formula: BFR = MAR X MS/BS, where MS is the mineralizing surface (double-labeled plus one half of the single-labeled surface) and BS is the bone surface. All histomorphometric formulae and nomenclature conform to the recommendations published by the American Society for Bone and Mineral Research (25). Bone biopsies were classified by the method of Ott et al. (7). In osteomalacia, OV/BV was >15% and peritrabecular fibrosis was <0.5% of the TV; in aplastic lesion, OV/BV was <15% and fibrosis was <0.5% of the TV. Both the osteomalacic and aplastic lesions were associated with a BFR reduced to less than 10 µm²/µm²/yr. In a mild lesion, OV/BV was <15% and fibrosis was <0.5% TV but BFR was relatively normal (more than 10 µm²/µm²/yr); in ostitis fibrosa, OV/BV was <15% and fibrosis was >0.5% TV and BFR was normal or high (more than 30 µm²/µm²/yr). Reference values for histomorphometric variables are shown in Table 2. Reported reference values for unselected uremic patients not yet undergoing dialysis treatment (26) are included to emphasize the wide spectrum of histology seen in the uremic syndrome relative to normal subjects.

STATISTICS
Simple statistical comparisons between the two groups for given variables were performed by using nonpaired t tests. The x² statistic was used to test for significance of symptom frequencies between the two groups. One-way analysis of variance was used to assess differences within the histological subgroups, for each parameter measured, followed by a posthoc statistic (the Newman-Keuls test, [27]) to determine intergroup P values in those histological parameters in which the F statistic indicated the probability of intergroup differences at a level of P ≤ 0.01.

RESULTS
Basic clinical and biochemical data relating to the two groups of patients are shown in Table 1. Bone
pain and clinical features of proximal myopathy were recorded as present or absent by the attending nephrologist at the time the patient was identified as having either hyperaluminemia or osteitis fibrosa. Bone pain and proximal myopathy were relatively common in both groups, although the frequency of these symptoms was not significantly different between them. The high serum aluminum group was significantly older ($P < 0.01$) and appeared to have undergone dialysis for a longer period of time, although this was not significant. The currently prescribed dose of Al(OH)$_3$ used as a phosphate-binding agent was not significantly different between the two groups. It was not, however, possible to calculate the total cumulative dose of Al(OH)$_3$ for individuals. The serum aluminum levels in the reference population of 196 hemodialysis patients were $2.7 \pm 2.1 \mu$mol/L (73 ± 57 µg/L). Serum aluminum levels in the osteitis fibrosa group were $2.5 \pm 2.1 \mu$mol/L (no different than those in the reference population but significantly lower [$P < 0.01$] than those in the hyperaluminemic group, whose serum levels were $7.8 \pm 3.4 \mu$mol/L). There was no correlation between serum aluminum levels and the currently prescribed dose of Al(OH)$_3$. The serum calcium, alkaline phosphatase, and PTH levels were all significantly higher in the osteitis fibrosa patients ($P < 0.01$).

There were no significant differences in bone volume (percent BV/TV) between Group 1 biopsies (22.8 ± 8.4) and those in Group 2 (29.0 ± 9.5). Within Group 1, the bone volumes were comparable within the subgroups (osteomalacia, 28.3 ± 10.4; aplastic lesions, 22.8 ± 8.8; osteitis fibrosa, 20.9 ± 4.8; and mild lesions, 18.7 ± 7.6). Table 2 shows the results of serum aluminum levels and the other histomorphometric measurements in the two groups. Also shown in Table 2 are reference values for both normal subjects and a cross-sectional analysis of unselected uremic patients with advanced renal failure (but not yet on dialysis), recently reported by Dahl et al. (26). These reference values were chosen because the authors also followed the conventions recommended by the American Society for Bone and Mineral Research (25). Significant differences between the subgroups are indicated in the footnotes to Table 2. Within Group 1 biopsies, there was a high prevalence (67%) of markedly impaired mineralization; nine patients had an aplastic lesion, and five had osteomalacia. Only one biopsy contained scanty occasional double tetracycline labels, allowing the calculation of the BFR, and in this patient, it was reduced to $12 \mu$m$^2/$µm$^2$/yr. Of the remaining seven biopsies, three showed osteitis fibrosa and four had only mild lesions; all contained double and single tetracycline labels, and BFR were well within the reference values reported by Dahl et al. (26) for unselected uremic patients. Aluminum deposits were identifiable in 17 (81%) biopsies by the Aluminon technique and in all 21 (100%) biopsies by the ASA method. Although the surface aluminum staining by the Aluminon technique was significantly higher in the osteomalacic lesions than in the aplastic, fibrotic, and mild lesions in Group 1 ($P < 0.05$), there were no overall differences between histological subgroups in surface aluminum identification by the ASA technique. The surface aluminum staining characteristics in the four histological subgroups are shown in Figure 1.

In Group 2 biopsies, the clinical evidence for severe secondary hyperparathyroidism was corroborated by the finding of extensive peritrabecular fibrosis in all biopsies, together with evidence for both vigorous resorption and osteoblastic function (Table 2)—all of which were significantly higher than corresponding values in Group 1 ($P < 0.05$). BFR were higher in this group than were BFR in the osteitis fibrosa subgroup of the biopsies from patients with higher serum aluminum, although not significantly so. Only 1 biopsy had surface staining for aluminum identified by the Aluminon technique (0.45% BS), but 11 (85%) had readily identifiable surface staining by the ASA stain that ranged from 1.25 to 49.2% BS (Figure 1). However, the surface ASA staining in this group was significantly less than that found in all of the subgroups within the high serum aluminum group ($P < 0.01$).

The operating characteristics of the two stains can be compared within the pooled data for all biopsies, divided into (1) the osteomalacic and aplastic lesions normally associated with aluminum toxicity and (2) all other subgroups. Within this sample, the sensitivity and specificity for the Aluminon stain were 93 and 75%, respectively. In contrast, the sensitivity for the ASA stain was 100% but the specificity was only 10%. Similarly, the positive predictive value (the probability of a positive test being associated with aluminum toxicity in bone) was high for the Aluminon stain (72%) but relatively low for the ASA stain (44%).

Table 3 shows a correlation matrix for selected variables pooled from both groups of patients. Serum aluminum levels correlate positively ($P < 0.01$) with surface ASA staining but not with surface Aluminon staining. There is also a significant negative correlation ($P < 0.01$) between serum aluminum and (1) indices of secondary hyperparathyroidism (bone surface osteoclast counts, $r = -0.43$; peritrabecular fibrosis, $r = -0.42$) and (2) indices of bone formation (osteoblast counts, $r = -0.45$; MAR, $r = -0.42$; and BFR, $r = -0.44$). Serum i-PTH levels as determined by a midmolecule RIA were negatively correlated with serum aluminum levels ($r = -0.50$; $P < 0.01$). There was also a significant negative correlation between the serum i-PTH assay and both histological stains for aluminum ($r = -0.41$ and $P < 0.05$ for Aluminon; $r = -0.61$ and $P < 0.01$ for ASA). Positive
TABLE 2. Bone histomorphometric indices in Group 1 (high serum aluminum) and Group 2 (osteitis fibrosa)\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Serum Aluminum (%)</th>
<th>Al/BS (%)</th>
<th>ASA/BS (%)</th>
<th>ES/BS (%)</th>
<th>NOc/Pm (No./cm)</th>
<th>Fibrosis (%TV)</th>
<th>OV/BV (%)</th>
<th>OTh (μm)</th>
<th>NOb/Pm (No./cm)</th>
<th>MAR (μm/day)</th>
<th>BFR (μm(^3)/μm(^2)/yr)</th>
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<tr>
<td><strong>Group 1</strong>&lt;br&gt;(High Serum Aluminum)</td>
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<tr>
<td>All Biopsies</td>
<td>21</td>
<td>7.8 ± 3.4</td>
<td>9.1 ± 12.5</td>
<td>63.5 ± 18.9</td>
<td>8.7 ± 5.8</td>
<td>2.3 ± 2.5</td>
<td>0.2 ± 0.6</td>
<td>9.7 ± 7.5</td>
<td>7.6 ± 2.8</td>
<td>4.8 ± 9.6</td>
<td>0.11 ± 0.19</td>
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<tr>
<td>Aplastic Lesion</td>
<td>9</td>
<td>8.0 ± 4.3</td>
<td>8.7 ± 12.7</td>
<td>67.4 ± 14.3</td>
<td>6.1 ± 4.1</td>
<td>1.8 ± 2.2</td>
<td>0</td>
<td>6.2 ± 4.9</td>
<td>6.7 ± 2.3</td>
<td>1.2 ± 2.6</td>
<td>0</td>
</tr>
<tr>
<td>Osteomalacia</td>
<td>5</td>
<td>8.2 ± 3.8</td>
<td>21.3 ± 15.7</td>
<td>68.2 ± 29.0</td>
<td>8.6 ± 5.3</td>
<td>2.0 ± 2.0</td>
<td>0</td>
<td>17.8 ± 2.6</td>
<td>10.2 ± 3.1</td>
<td>3.0 ± 4.9</td>
<td>0</td>
</tr>
<tr>
<td>Osteitis Fibrosa</td>
<td>3</td>
<td>7.5 ± 1.7</td>
<td>0.2 ± 0.3</td>
<td>55.9 ± 4.1</td>
<td>16.0 ± 5.4</td>
<td>5.8 ± 2.3</td>
<td>1.3 ± 1.1</td>
<td>16.4 ± 10.7</td>
<td>9.3 ± 3.0</td>
<td>21.8 ± 16.7</td>
<td>0.31 ± 0.10</td>
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<tr>
<td>Mild Lesion</td>
<td>4</td>
<td>6.9 ± 2.4</td>
<td>4.7 ± 3.3</td>
<td>55.8 ± 25.3</td>
<td>8.9 ± 6.8</td>
<td>0.9 ± 1.8</td>
<td>0</td>
<td>4.5 ± 2.7</td>
<td>6.0 ± 1.3</td>
<td>2.0 ± 4.0</td>
<td>0.30 ± 0.28</td>
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<tr>
<td><strong>Group 2</strong>&lt;br&gt;(Osteitis Fibrosa)&lt;br&gt;(Reference Values)</td>
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<tr>
<td>Normal Subjects</td>
<td>&lt;0.5</td>
<td>0</td>
<td>0</td>
<td>3.0 ± 0.9</td>
<td>7.5 ± 4.8</td>
<td>0</td>
<td>3.3 ± 1.7</td>
<td>8.7 ± 2.0</td>
<td>NA</td>
<td>0.53 ± 0.06</td>
<td>13.1 ± 10.6</td>
</tr>
<tr>
<td>Uremic Patients</td>
<td>&lt;0.5</td>
<td>0</td>
<td>0</td>
<td>10.9 ± 7.7</td>
<td>NA</td>
<td>0</td>
<td>6.6 ± 5.8</td>
<td>9.6 ± 3.3</td>
<td>NA</td>
<td>0.56 ± 0.11</td>
<td>22.6 ± 19.7</td>
</tr>
</tbody>
</table>

\(^a\) Data are mean ± SD. NA, not available; OTh, osteoid seam thickness.

\(^b\) P < 0.05; cf. all other subgroups.

\(^c\) P < 0.05; cf. all other subgroups in Group 1 except osteitis fibrosa.

\(^d\) P < 0.05; cf. Group 1.

\(^*\) P < 0.05; cf. all subgroups in Group 1.

\(^1\) From Dahl et al (26).

\(^2\) From Erviksen et al (39).
correlations were found between serum i-PTH and the indices of bone resorption and formation shown in Table 3 (P < 0.01).

There was no correlation between either of the surface aluminum stains and other measurements of bone osteoid matrix. The two staining methods for aluminum deposits correlate well (r = 0.57; P < 0.01).

There is a consistent negative correlation (P < 0.01) between surface aluminum deposits identified by ASA stain and bone resorption indices, including the total eroded surface (r = -0.68), osteoclast counts (r = -0.53), and peritrabecular fibrosis (r = -0.47). The correlation between the eroded surface (percent ES/BS) and surface ASA stain is shown in Figure 2. The same relationships do not hold for surface Aluminon staining.

There were also strong negative correlations (P < 0.01) between the ASA stain and several indices of bone formation including surface osteoblast counts (r = -0.61), MAR (r = -0.63), and BFR (r = -0.64). These are shown graphically for the surface osteoblast counts and BFR in Figure 3. Again, these relationships were not seen for the Aluminon stain. There were no correlations between aluminum stain and osteoid volume, thickness, or surface extent.

When these correlations were made separately, within Groups 1 and 2, the pattern of significant correlations between serum aluminum and the histological variables was obscured, largely because the histological findings in aluminum-associated bone disease tend towards one end of the spectrum (very low bone turnover, with little evidence for bone resorption and low bone formation). Thus, serum aluminum was significantly correlated with the ASA stain in the osteitis fibrosa group only (r = 0.59; P < 0.01). In neither group were the positive correlations maintained between serum aluminum and the following histological measurements: NOc/Pm, fibrosis, NOb/Pm, MAR, BFR. The Aluminon stain was positively correlated with the ASA stain in the high serum aluminum group only (r = 0.53; P < 0.01); however, this stain was essentially negative in the osteitis fibrosa group. The significant correlations with the ASA stain were preserved within the high serum aluminum group for ES/BS (r = -0.44), MAR (r = -0.64), and BFR (r = -0.44) only. Although the directional trends for the correlations between the ASA stain and histological measurements were found in

![Figure 1](image)

Figure 1. Surface staining of aluminum deposits identified by the Aluminon stain and the ASA stain. Individual biopsy results are represented by histological subgroup within the hyperaluminemic patients (Group (Gp) 1) and separately for the control group of osteitis fibrosa (OF) patients (Group 2) OM, osteomalacia; ApI, aplastic lesion; Mild, mild lesion.

<table>
<thead>
<tr>
<th>TABLE 3. Correlation coefficients (r) for serum aluminum levels and selected histomorphometric variables</th>
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<tr>
<td>Serum Aluminum</td>
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<tr>
<td>Serum Aluminum</td>
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<tr>
<td>Al/BS</td>
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<tr>
<td>ASA/BS</td>
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<tr>
<td>ES/BS</td>
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<td>NOc/Pm</td>
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<td>Fibrosis</td>
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<td>NOb/Pm</td>
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<td>MAR</td>
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<td>BFR</td>
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*Significance levels: for r ≥ 0.35, P < 0.05; for r ≥ 0.45, P < 0.01. NS, not significant.
DISCUSSION

In this study, serum aluminum levels in excess of 5.0 μmol/L (135 μg/L) had a 67% positive predictive value to detect either osteomalacia or aplastic bone lesions (14 out of 21 biopsies) in chronically hemodialyzed patients. However, many patients with these two bone lesions, which are usually associated with aluminum toxicity, would be missed if this were the only selection criterion used. In fact, the true prevalence of osteomalacia and aplastic lesions in hemodialysis patients can only be assessed from a cross-sectional series of unselected biopsies. In two cross-sectional series in which the classification of renal osteodystrophy was comparable to that used here, Sebert et al. (28), and Llach et al. (10) reported the underlying prevalence as 16 and 32%, respectively. In this article, 14 patients from among 196 chronically hemodialyzed subjects were identified with these bone lesions as a result of detecting hyperaluminemia. Assuming that the underlying bone histology in the several hyperaluminemic patients who did not undergo a bone biopsy was similar to that in those who were biopsied, a serum aluminum level of ≥5 μmol/L would identify a prevalence of osteomalacia or aplastic bone disease equivalent to 9.5% of the population (only about half the expected prevalence). More cross-sectional information about bone histology in patients with lower serum aluminum levels is required in order to determine the true sensitivity of hyperaluminemia to detect these bone lesions within our dialysis population. Although we do not have systematic bone histology from our population, we have estimated in an earlier publication that this degree of hyperaluminemia has a sensitivity of 60% when the indication for obtaining a bone biopsy was the clinical presentation of bone pain or dementia (16). However, the relatively high predictive value of this cutoff level for serum aluminum suggests two useful clinical guides: (1) in symptomatic patients, hyperaluminemia is a strong impetus to proceed to a bone biopsy in order to confirm a histological diagnosis of aluminum-related osteomalacia and the need for chelation therapy; and (2) in asymptomatic patients, hyperaluminemia is a strong indication to discontinue aluminum hydroxide-based phosphate-binding agents.

The dialysis patients with severe osteitis fibrosa, who represented a "control" group in this study, had baseline levels of serum aluminum that were similar to those of the underlying dialysis population mean, and only one patient had small amounts of aluminum deposits identified by the Aluminon stain. The absence of an apparent association between bone alu-
minum accumulation and ostettis fibrosa has been reported many times (7,9,10,17,29), and for this reason, we chose such a patient group as a control while evaluating the ability of the ASA stain to detect bone aluminum deposits. However, 11 of the 13 controls were positive by the ASA stain and all of the biopsies in the hyperaluminemic patients were strongly positive. In a technical note on the ASA stain, Kaye et al. (11) also noted that surface aluminum identification by this method was usually much more extensive than that identified by the Aluminon method. As in the study presented here, Kaye et al. found a positive ASA stain in all histological subgroups (11). Kaye et al. also found strong correlations between chemically extractable bone aluminum content and surface staining by both techniques and concluded that the ASA technique is a more sensitive technique for identifying past aluminum exposure. Our data, when applied to the selective indices of either hyperaluminaemia or severe ostettis fibrosa, confirm the significant relationship between the two stains ($r = 0.56$), as well the more extensive staining by the ASA method in all bone lesions compared with the Aluminon stain. When considered simply as diagnostic tests to identify osteomalacia and aplastic lesions, the Aluminon stain has a relatively useful sensitivity (93%) and specificity (75%); although the ASA stain has 100% sensitivity, the large number of false-positive values leads to a very low specificity (10%). Therefore, the relatively nonspecific staining characteristics of the ASA stain limit its utility as a diagnostic tool to identify aluminum dependent bone disease (i.e., dialysis osteomalacia and aplastic lesions); at present, the clinical implications of a positive ASA stain might simply be limited to a reflection of past or current aluminum exposure.

The ASA-stained surface aluminum deposits found in the two groups of selected biopsies in this article were quite strongly but negatively correlated with several histological parameters of hyperparathyroidism including peritrabecular fibrosis ($r = -0.47$), surface osteoclast counts ($r = -0.53$), and total cancellous eroded surface ($r = -0.68$). These relationships did not hold for Aluminon-stained surfaces, in part because this stain is usually negative in uncomplicated ostettis fibrosa, and thus, the range of values within which to make the correlations is more restricted. It is clear from previous clinical studies that PTH modulates the interactions between aluminum accumulation and the evolution of impaired mineralization in renal osteodystrophy. Aluminum accumulates in parathyroid glands (30), and serum PTH levels in dialysis patients appear to be suppressed as a consequence of aluminum toxicity because removal of the source of aluminum exposure (31) or chelation therapy with DFO (32,33) results in a gradual increase in PTH levels. It is often assumed that high levels of circulating PTH are protective at the skeletal level because bone aluminum content is usually lower in patients with ostettis fibrosa (29); surface staining for aluminum by the Aluminon technique is lowest in groups of biopsies with "pure" ostettis fibrosa (7), whereas conversely, parathyroidectomy leads to accelerated aluminum deposition on cancellous bone surfaces (20). Moreover, most patients with dialysis osteomalacia are functionally hypoparathyroid (21,29,34). The significant negative association between the ASA-stainable aluminum in this study and both "immunoassayable" serum PTH levels and histological parameters of hyperparathyroidism supports the concept that increased PTH activity protects against the development of aluminum-induced bone toxicity. However, the corollary, namely that increasing aluminum exposure leads to inhibition of PTH secretion and/or action, may be just as true.

There was a strong negative correlation ($r = -0.61$) between the surface aluminum identified by the ASA stain and the osteoblast counts identified over the osteoid surfaces. This correlation did not hold for the Aluminon stain, and there were no correlations observed between either stain and other measured osteoid indices, specifically, the active formation surfaces (osteoid seams lined by cuboidal osteoblasts). The association between aluminum deposits identified by the Aluminon stain and either total osteoblast counts or active formation surfaces has been noted in several previous studies of relatively unselected biopsies from hemodialysis patients (35-38). Again, the absence of such a correlation in our study probably reflects the inclusion of a significant proportion of ostettis fibrosa cases, in which the Aluminon stain is negative. However, the increased sensitivity of the ASA stain to detect aluminum uncovers the association and further supports the hypothesis that aluminum accumulation in bone has both a deleterious effect on osteoblast cell numbers as well as a putative action to impair bone mineralization.

The significant negative correlations between all three measurements of aluminum toxicity (serum aluminum and Aluminon and ASA stains) and MAR or BFR are corroborated by several other reports linking aluminum accumulation to impaired mineralization in dialysis patients. However, the correlations are strongest for the ASA stain compared with the other two indices ($r = -0.6$ versus $-0.4$; Table 3).

In conclusion, this study demonstrates the useful predictive values for a serum aluminum level $\geq 5 \mu mol/L$ to identify dialysis patients with either dialysis osteomalacia or aplastic bone lesions—the two variants of renal osteodystrophy most clearly identified with aluminum toxicity. At the very least, serum aluminum levels exceeding this value provide strong clinical grounds to discontinue aluminum-based phosphate-binding agents. In applying the ASA stain to the spectrum of renal osteodystrophy, it would appear that this stain has a much lower diagnostic
specificity to identify what is conventionally described as "aluminum-associated" bone disease. Rather, the ASA stain identifies aluminum deposits throughout the spectrum of renal osteodystrophy and presumably reflects the past or current exposure to aluminum. However, the negative associations between bone cellular functions, MAR, and BFR on the one hand and the ASA-stained aluminum on the other are certainly consistent with a toxic role for aluminum in all forms of renal osteodystrophy.

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