PART X

BONE DISEASE

Chairman: Dr E Ritz
Skeletal Lesions and Calcium Metabolism in Early Renal Failure

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Introduction

Relatively little is known about the bone lesions in incipient renal insufficiency (Malluche et al, 1974). This is somewhat surprising, since it has been postulated (Shaldon et al, 1973) that prophylaxis of renal bone disease should begin in the early asymptomatic stages of the disease.

This concept, though reasonable, is not based on firm knowledge of what happens in the skeleton. It is therefore the purpose of the present investigation to examine in a cross-sectional type of study 50 patients with chronic renal disease at various levels of GFR. In the following communication an analysis of serum biochemistry and bone histology for these patients is given.

MATERIAL AND METHODS

Fifty patients with chronic renal disease (duration ranging from one month to seven years) were examined. All patients were subjected to tetracycline double labelling: they were given tetracycline hydrochloride 500 mg/24 hr from days 18 to 16 and 4 to 0 prior to the biopsy. Iliac crest biopsies were taken with an electric drill (Shaldon et al, 1973). The biopsies were fixed in alcohol; undecalciﬁed sections, embedded in methylmethacrylate were stained after Masson Goldner and evaluated quantitatively by micromorphometry as described previously (Ritz et al, 1971). All sections were analysed under polarised light for measurements of woven osteoid. Fluorescence microscopy was performed on unstained sections and the fraction of osteoid seams, taking tetracycline label in a double front, was counted. Serum-PTH was measured by radio-immunoassay. Serum-Ca, P and alkaline phosphatase were measured by autoanalyser, ionised serum-Ca with the Orion electrode, urinary hydroxyproline according to Firschein
(1966) and bone phosphatase after electrophoretic separation on cellulose acetate (Seiffert et al, in preparation). GFR was measured as creatinine clearance with standard techniques. Statistical analysis was carried out using a digital computer-system (LAB 11).

RESULTS AND DISCUSSION

Bone Histology

As shown in Table I, bone abnormalities can be recognised in the earliest stages of renal insufficiency.

(a) Woven Osteoid

Woven osteoid was seen in some patients with a GFR > 80 ml/min · 1.73 m² (Figure 1). The fraction of osteoid seams consisting of woven osteoid increased with decreasing GFR. In patients with early renal failure, woven osteoid was rare and spotty. It was found filling up osteoclastic resorption defects, so that resorbed premorbid lamellar bone was replaced by poorly mineralised woven bone matrix. However, extended apposition fronts consisting of woven osteoid were not seen before the GFR fell below 30 ml/min · 1.73 m².

(b) Mineralisation Defect

A mineralisation defect was defined as an increase of the fraction of lamellar osteoid seams failing to take up tetracycline label with the double-labelling technique. (Since woven osteoid does not usually mineralise with a distinct mineralisation front, osteoid recognisable as woven osteoid was omitted when counting mineralising osteoid seams). Although individual patients at a GFR over 40 ml/min had an increased fraction of non-labelled osteoid seams (Figure 2), few patients with mineralisation defects were seen before GFR fell below 40 ml/min · 1.73 m². Osteoid, accumulating in patients without mineralisation defect, is due to an increased birthrate of osteoid seams (Frost, 1963) rather than to their prolonged lifespan as found in patients with defective mineralisation. This is reflected by the finding (Table I) that the relative increase of the fraction of trabecular surface covered by osteoid seams (OS) was greater than the relative increase of the width of osteoid seams (S); ie osteoid increased in extent rather than in width.

(c) Abnormal Histology with GFR > 70 ml/min · 1.73 m²

In 2 out of 10 patients osteoid (OS) was definitively outside of the normal range. Osteoclasts were not increased in these patients, although inactive shallow resorption zones (‘flache resorption’) were abundant.
TABLE I. Bone histology at various levels of GFR

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 12)</th>
<th>&gt; 60 (n = 10)</th>
<th>60–41 (n = 13)</th>
<th>40–21 (n = 12)</th>
<th>&lt; 20 (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_o ) (% of spongiosal volume)</td>
<td>17.1 ± 2.4</td>
<td>16.4 ± 3.76</td>
<td>21.6 ± 2.25</td>
<td>22.6 ± 1.57</td>
<td>20.2 ± 1.59</td>
</tr>
<tr>
<td>( V_o ) (% of spongiosal volume)</td>
<td>0.18 ± 0.09</td>
<td>0.19 ± 0.12</td>
<td>0.78 ± 0.22</td>
<td>2.28 ± 1.03</td>
<td>1.98 ± 0.442</td>
</tr>
<tr>
<td>( S/V ) (mm²/mm³)</td>
<td>17.3 ± 1.6</td>
<td>18.7 ± 4.0</td>
<td>17.1 ± 1.53</td>
<td>16.1 ± 0.84</td>
<td>18.7 ± 1.1</td>
</tr>
<tr>
<td>OS (% of trabecular surface)</td>
<td>9.6 ± 3.4</td>
<td>9.1 ± 4.4</td>
<td>22.6 ± 4.3</td>
<td>40.6 ± 5.87</td>
<td>49.7 ± 7.16</td>
</tr>
<tr>
<td>( \xi ) (μ)</td>
<td>7.5 ± 2.8</td>
<td>6.4 ± 4.2</td>
<td>9.65 ± 1.4</td>
<td>11.5 ± 3.26</td>
<td>9.86 ± 1.1</td>
</tr>
<tr>
<td>HO (% of nonosteoid trabecular surface)</td>
<td>0.95 ± 0.3</td>
<td>0.9 ± 0.14</td>
<td>1.45 ± 0.17</td>
<td>2.64 ± 0.25</td>
<td>4.24 ± 6.52</td>
</tr>
<tr>
<td>EOF (% of trabecular surface)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.4 ± 1.37</td>
<td>8.1 ± 1.67</td>
</tr>
</tbody>
</table>

(d) Abnormal Histology with Normal Serum PTH-levels

In 5 of 16 patients with normal serum-PTH (< 200 pgEq bov-PTH/ml) at a GFR < 70 ml/min • 1.73 m², increased numbers of osteoclasts were counted. Although problems with the measurement of PTH can certainly not be excluded, the finding may also point to the fact that single PTH measurements are not representative of average serum PTH activity especially in early renal failure since PTH levels fluctuate widely throughout the day.

(e) Bone Histology at Various Levels of GFR

Osteosclerosis, i.e. an increase of the fraction of spongiosal volume represented by mineralised bone, was seen first in patients with a GFR < 60 ml/min • 1.73 m².

The increase of \( V_o \) correlated inversely with serum-Ca \((r = -0.29)\) and positively
with the accumulation of osteoid ($V_0$) ($r = 0.43$). Increased cancellous bone mass is thus the consequence of an increase of osteoid.

*Accumulation of osteoid* was demonstrated by measuring the extent of trabecular surface covered by osteoid seams (OS), the fraction of spongiosal volume represented by unmineralised bone matrix ($V_0$) and by calculating the average seam thickness ($\bar{S}$). The increase of OS correlated significantly with the fall of GFR ($r = -0.6$) and the increase of PTH ($r = 0.612$). Osteoid volume correlated with the presence of lower arterial pH ($r = -0.36$), pointing to the higher degree of acidosis in hyperparathyroid patients with increased osteoid.

Osteoid (OS) was correlated with osteoclastic surface resorption (HO) ($r = 0.79$) and endosteal fibrosis (EOF) ($r = 0.69$) pointing to the tight coupling between bone apposition and bone resorption. The ratio of trabecular surface to trabecular volume (S/V) which is inversely related to trabecular diameter, correlated with osteoid seam thickness ($S$) ($r = -0.5$) illustrating that trabeculae are coarse as a consequence of the osteoid coating.

Both *osteoclastic surface resorption* ($r = 0.63$) and *endosteal fibrosis* ($r = 0.76$) correlated with serum-PTH and ionised serum-Ca. (HO with ionised Ca; $r = -0.43$; EOF with ionised Ca; $r = -0.39$; EOF was not seen before GFR fell below 30 ml/min $\cdot$ 1.73 m$^2$. HO rose as GFR fell ($r = -0.60$).
B. Serum Biochemistry as an Indicator of Bone Histology

(a) Serum-Ca

Total serum-Ca and ionised serum-Ca were correlated and changed in parallel ($r=0.65$); it was not before GFR fell below 20 ml/min $\cdot$ 1.73 m$^2$ that the correlation between ionised serum-Ca and total serum-Ca and was no longer significant ($r=0.36$). At this level of GFR, serum protein levels fall and in addition complexed Ca rises at the expense of ionised serum-Ca. Consequently, little diagnostic accuracy is gained by measuring ionised serum-Ca in early renal failure, whereas below GFR 20 ml/min $\cdot$ 1.73 m$^2$, ionised serum-Ca can no longer be safely predicted on the basis of total serum-Ca. Interestingly, total serum-Ca in terminal renal failure correlated better ($r=-0.69$) than ionised serum-Ca ($r=-0.56$) with osteoid volume ($V_0$); since in terminal uraemia, total serum protein decreases, lowering of serum-Ca due to the decrease of serum protein is superimposed upon the decrease of serum-Ca due to uraemia, resulting in a spuriously better correlation. As a result, the patient with hypocalcaemia is more likely to have increased osteoid in his skeleton (Ritz et al 1973). Osteoclastic surface resorption (HO) increased as ionised serum-Ca decreased ($r=-0.43$);
thus, osteoclastic surface resorption is stimulated by hyperparathyroidism secondary to hypocalcaemia. Evidently, increased surface resorption is unable to bring serum-Ca levels back into the normal range.

(b) Alkaline Serum Phosphatase

Serum alkaline phosphatase and specific bone phosphatase did not correlate with serum-PTH levels. They did, however, \((r = 0.76)\) correlate with osteoid volume \((V_o)\). The lack of correlation is hardly surprising, since osteoid volume \((V_o)\) correlated but loosely \((r = 0.45)\) with serum-PTH. Thus, serum alkaline phosphatase and specific bone phosphatase, while reflecting skeletal changes, are rather poor indicators of serum-PTH levels in early renal failure.

Although total serum alkaline phosphatase and specific alkaline bone phosphatase were closely correlated \((r = 0.94)\), the discrimination between normal and abnormal findings was different, total serum alkaline phosphatase being abnormal in 16% and specific bone phosphatase being abnormal in 77% of the patients. Serum alkaline phosphatase and specific bone phosphatase correlated equally well with various parameters of bone histology.

(c) Urinary-Ca Excretion

As GFR decreased, so urinary excretion of Ca fell \((r = 0.44)\). The decrease occurred no later than at GFR of 80 ml/min · 1.73m². Low urinary-Ca was correlated with low ionised serum-Ca \((r = 0.508)\). Although urinary excretion of Ca was low in absolute terms, it served as a sensitive indicator, predicting vitamin-D intoxication up to three weeks ahead of hypercalcaemia — even in patients with a GFR < 5 ml/min · 1.73 m² (Mehls and Ritz, in preparation).

(d) Urinary Hydroxyproline

Total urinary hydroxyproline (measured after Firschein), an indicator of bone matrix turnover, did not correlate at all with either bone histology or alkaline phosphatase.

(e) Metabolic Acidosis

Arterial pH correlated \((r = -0.36)\) with osteoid volume \((V_o)\). This does not necessarily mean that mineralisation of osteoid is inhibited by acidosis, but may merely reflect the well-known tendency of hyperparathyroid subjects to be in metabolic acidosis — be it due to transfer of \(H^+\) across cellular or subcellular membranes of increased \(H^+\) equivalent generation by bone-mineral precipitation in high-turnover bone disease.

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Figure 3. Correlation between GFR and PTH.

(f) Serum-PTH Levels

Fasting serum-PTH levels were increased (> 200 pgEq bov-PTH/ml) in 1 of 7 patients with GFR > 70 ml/min • 1.73 m² and in 26 of 42 patients with GFR < 70 (Figure 3). Serum-PTH increased with decreasing GFR (r = – 0.56) and was significantly correlated with the fractional phosphatase clearance (Cₚ/Cₜₐₚ) (r = 0.51). The rise of serum-PTH followed a hyperbolic curve, similar to what is seen for urinary excretion products such as creatinine. Serum-PTH rose as ionised serum-Ca fell (r = – 0.54). This close correlation suggests that in early renal failure stimuli for PTH secretion other than ionised Ca do not play a recognisable role.

There was no correlation between serum-PTH levels and alkaline serum phosphatase or alkaline bone phosphatase. This indicates, that the intensity and/or duration of hyperparathyroidism (Ritz et al, 1974) is not sufficient in early renal failure to elicit a bone tissue response in all cases.

Conclusion

Skeletal abnormalities appear in the earliest stages of renal insufficiency. Evidence of parathyroid hormone overactivity in the skeleton is almost obligatory, while deficient mineralisation occurs only in a minority of patients.
References


Ritz, E, Malluche, H H, Bommer, J, Mehls, O & Krempien, B (1974) Nephron, 12, 393

Open Discussion

J F MOORHEAD (London) There was a slide of endosteal fibrosis which suddenly starts to be obvious at a GFR of about 30 ml/min. Do you know why there is such a sharp change?

MALLUCHE I can only speculate, but endosteal fibrosis is a histological indicator for PTH over-secretion.

E RITZ (Heidelberg) So what you are implying is that this is a threshold phenomenon: beyond a certain level of PTH endosteal fibrosis appears giving evidence of an activation of endosteal cells.

J W COBURN (Los Angeles) Have you done any correlations when the GFR is 50–60? Also although you say that there is evidence of secondary hyperthyroidism early on, PTH and endosteal fibrosis were normal, although I did not recall what you said about osteoclastic resorption surface. Woven bone was abnormal in only one of two of these, so what do your data really show? You seem to suggest that only a small fraction show abnormalities.

MALLUCHE We have not studied small ranges of renal function, so the detailed information is not available. The problem with PTH is that it fluctuates throughout the day, and our single measurements may be misleading.

COBURN What fraction of the patients with GFR’s above 60 showed increased resorptive surface?

MALLUCHE About one-third of patients with GFR > 50 ml/min had evidence of overactivity of osteoclasts, either present or past (woven osteoid).