Acta orthop. scand. 48, 566–571, 1977

OSTEOCLAST COUNTING IN CRISTA BIOPSIES

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A description is given of a modified method of counting osteoclasts both per section area and also relative to the remaining trabeculae surface area in crista biopsies. The material consisted of normal individuals, patients with clinical osteoporosis, and patients with chronic renal failure undergoing haemodialysis. The number of osteoclasts in the biopsies from normal and osteoporotic individuals showed a normal distribution with the same mean. In the haemodialysis patients there was a marked skew distribution. In normal individuals, there was a significant decrease in the number of osteoclasts per section area with age, but this was not significant when calculated relative to the bone surface area.

Key words: crista biopsies; osteoclasts; osteoporosis; haemodialysis; renal osteopathy

Accepted 10.vi.77

When looking through histological slides from crista biopsies of older normal or osteoporotic humans, an almost total absence of osteoclasts (and osteoblasts) is apparent. Therefore, it is difficult to explain how the bone tissue can disappear with increasing age, especially in postclimacteric women. The periosteocytic resorption described by Belánger (1971) cannot be valid in "idiopathic" osteoporosis since the remaining bone trabeculae always have normal bone density. Jaffe (1972), among others, considers therefore that the osteoclasts do not have an exclusive significance in the development of osteoporosis.

Until the opposite is proven, however, there is reason to consider osteoclasts as being responsible for bone resorption also in the development of osteoporosis. It is possible that osteoporosis develops very rapidly, mainly in the initial period after menopause, or that some types of osteoporoses proceed stepwise by repeated osteoclast attacks over short periods. In fact, during the last few years some substances producing osteoclastic resorption of bone have been investigated, e.g., OAF (osteoclast activating factor), produced *in vitro* by mitogenic stimulation of lymphocytes (Horton et al. 1972) and prostaglandins (Dietrich & Raisz 1975), active, for example, in connection with immune response and inflammatory disease (and malignancy). These and perhaps other substances with similar effect, working over short periods, could explain the apparent defective connection between bone resorption and acting cells.

Financial support was obtained from Herman Järnhardts Stiftelse and the Swedish Medical Research Council.

It might, therefore, be of value to have an easy and reproducible method for counting osteoclasts per surface area of bone. The determination of active and inactive resorption surfaces and bone formation surfaces used earlier is more difficult and perhaps not so useful when testing the hypotheses mentioned. Data in the literature concerning the normal number of osteoclasts are sparce and vary greatly and the number has been decided per area of the section (e.g., Bordier & Tunchot 1972, 0.37/mm^2, and Ellis & Peart 1973, 0.01–0.03/mm^2). In this paper we have tested a modified method for counting osteoclasts both per area of section and per surface of bone area in normal individuals compared with patients with osteoporosis and patients with chronic nephropathy undergoing haemodialysis.

MATERIAL AND METHODS

The control group consisted of 51 men and 36 women (10–90 years) who had died suddenly following cardiac arrest, accident or suicide and had been brought to the Forensic Department for autopsy. A wedge-shaped sample was taken from the iliac crest just dorsal to the iliac spine.

The osteoporosis group consisted of 48 women (44–71 years) with radiological and clinical signs of spinal osteoporosis. The nephropathic group consisted of 91 patients, all of them undergoing haemodialysis. Details of the nephropathic patients with data relating to the state of the disease and laboratory examinations will be presented in another paper. In the present paper we have only used the biopsies for testing the osteoclast counting method.

A 15 × 3 mm cylindrical sample was obtained from the patients with a motor-driven slow moving drill according to Burckart. The sample was taken vertically about one cm dorsal to the iliac spine.

The samples were fixed in formalin, dehydrated in alcohol-chloroform, and embedded in methylmethacrylate without previous decalcification and finally cut into sections (4–7 μ). The sections were stained according to Goldner (1938).

The margins of the sections were taped in order to obtain a straight-lined rectangular area of trabecular bone. All osteoclasts in this area were counted (per mm^2) with 40 × objective and 10 × eyepiece magnification. The osteoclasts were identified by their light pink, often vacuolar, cytoplasm, and the distinct nucleoli. Only osteoclasts with two or more nuclei and in contact with the bone surface were counted.

In our evaluations of trabecular and osteoid surfaces, we used an objective magnification of 13 × and an eyepiece magnification of 10 ×. We used a grid with wave-formed lines in the eyepiece as described by Schenk et al. (1969). We counted the number of intersections between the grid lines and trabecular and osteoid surfaces in 25–30 fields of every specimen. In order to make the whole procedure simpler, we counted only the number of intersections without calculating the trabecular circumference area as in Schenk et al. (1969). This latter calculation does not give any further useful information beyond the counted data, and our main interest is to present the number of osteoclasts relative to the remaining bone tissue, which can be used to compare groups of specimens with each other.

RESULTS

The number of osteoclasts per section area and the number relative to the bone surface area in normal individuals, in osteoporotics and in haemodialysis patients, are seen in Table 1. The mean of the absolute and the relative number of osteoclasts is the same in normal and osteoporotic individuals; in haemodialysis patients almost ten times higher. The frequency distribution of osteoclasts per mm^2 section in per cent of the total number of normal individuals, osteoporotics and haemodialysis patients, respectively, is shown in Figure 1. It seems clear that 99 per cent of the normal individuals have between 0–0.1 osteoclasts per mm^2 section. Most (or 90 per cent) of the osteoporotic patients have the same average number of osteoclasts as normal individuals but there is also a small group with between 0.1–0.2 osteoclasts. It is remarkable that so many of the osteoporotic patients (30 per cent) have no osteoclasts at all. In haemodialysis patients the frequency distribution is skew.

In the normal and in the dialysis group the osteoclast count/mm^2 decreased sig-

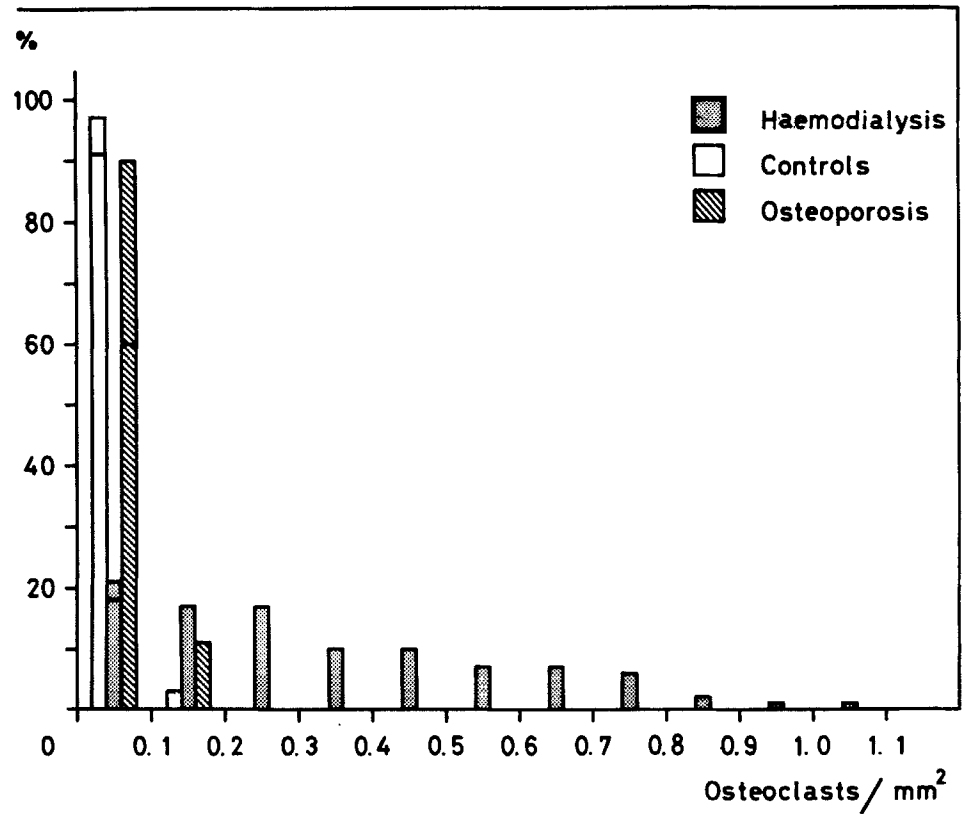

Figure 1. Histogram showing the frequency distribution of the number of osteoclasts/mm² as a percentage of the total number of normal individuals and patients with osteoporosis or haemodialysis. The detached upper parts of the three staples in the 0–0.1 area represent cases without osteoclasts.

Table 1. The number of osteoclasts per mm² of the section and the number of osteoclasts relative to the bone surface area in the total material. The correlation coefficients for the number of osteoclasts relating to age are also shown.

		Osteoclasts/mm² section		Osteoclasts relative to bone surf. area	
		Mean and SD	Age regression	Mean and SD	Age regression
Normal	$n = 87$	0.04 ± 0.02	r = —0.32**	0.003 ± 0.001	r = —0.19
Osteoporosis	$n = 48$	0.04 ± 0.04	r = —0.19	0.003 ± 0.003	r = —0.23
Haemodialysis	$n = 91$	0.32 ± 0.26	r = —0.37**	0.024 ± 0.014	r = —0.27*

** = $0.01 > P > 0.001$ * = $0.05 > P > 0.01$

nificantly with age. See Table 1 and Figure 2 (normal individuals). There was no difference between men and women. However, when the osteoclast numbers were divided by the relative bone surface area, the age decrease was no longer significant for normal individuals, but there was a low significance for the haemodialysis group. See Table 1 and Figure 3 (normal individuals). The correlation between the number of osteoclasts/mm² and the relative bone surface

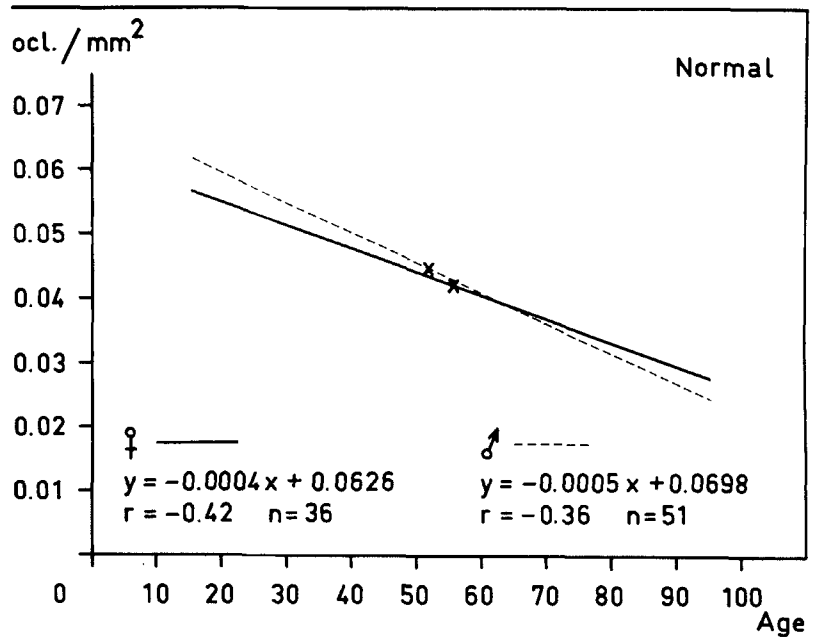

Figure 2. The relation between the number of osteoclasts per mm² section and the age of normal individuals. There is a significant correlation in both sexes. The correlation coefficient for both sexes r = —0.32.

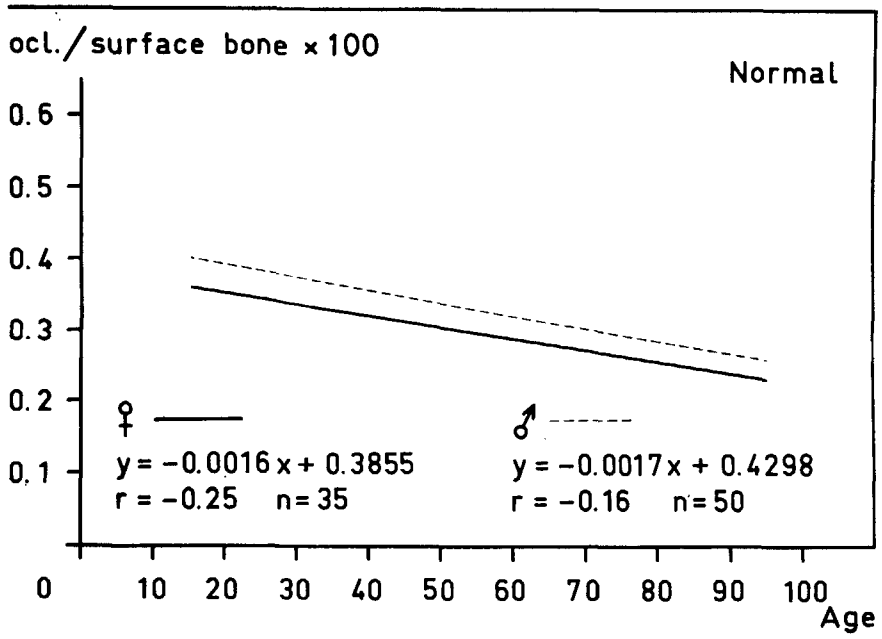

Figure 3. The relation between the number of osteoclasts per surface bone and the age of the normal individuals. There is no longer any significant correlation.

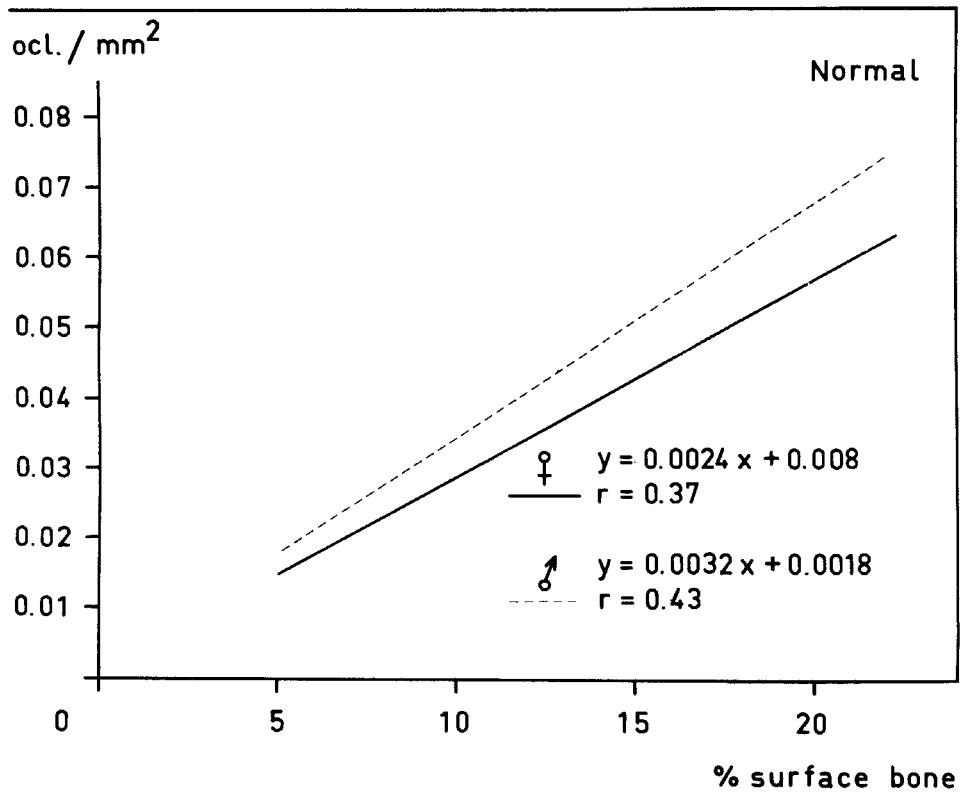

Figure 4. The correlation between the number of osteoclasts counted per mm² section of the slides and the percentage surface area of bone. There is a significant correlation.

area in normal (Figure 4) and dialysis cases was significant. There was, however, no correlation between osteoid surface and the number of osteoclasts/mm².

DISCUSSION

A method for counting osteoclasts in the iliac crest is described which differs only slightly from previous methods. The average number of osteoclasts is very low in sections from normal and osteoporotic iliac crests, and lower than that presented by Bordier & Tunchot (1972). The average osteoclast number in patients undergoing haemodialysis was almost ten times higher, but the frequency distribution, unlike normal and osteoporotic individuals, is quite skew—perhaps indicating different degrees of severity of the disease.

This difference between our normal values and those of Bordier & Tunchot might be due to our not having counted osteoclasts with only one nucleus, either actual uni-nuclear or multinuclear cells cut through one pole. Our way of counting is easier and certainly more reproducible. It is not greatly dependent on the quality of staining.

In the normal group we found a decreasing osteoclast count/mm² of the section with age and no difference between men and women. This decrease is not actual, being due to decreased bone surface, which was also found by Schenk et al. (1969). In five cases in the osteoporosis group, we found a large number of osteoclasts (0.1–0.2/mm²), out of

keeping with the rest of the group. Most of these osteoclasts were lying close to the surface of the trabeculae without Howships lacunae. We have no information as to whether these patients were suffering from some illness. Thus, we have no explanation for this finding other than it might possibly involve cases where the bone tissue was exposed to an incipient osteoclastic attack.

There was no correlation between the number of osteoclasts and the osteoid surface, which supports the hypothesis that osteoclasts resorb the bone tissue intermittently.

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