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Differential patterns of osteoblast dysfunction in trabecular bone in patients with established osteoporosis

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Abstract

***Aims*—To analyse osteoblast function in 153 cases of established osteoporosis as previous work has indicated that osteoporosis is a heterogeneous condition characterised by different patterns of osteoclast and osteoblast dysfunction.**

***Methods*—Histomorphometric data from 153 cases with established osteoporosis was used to analyse osteoblast function, using the following parameters: osteoblast number was assessed using the ratio of osteoblast surface to bone surface (ObS:BS); the percentage of active osteoblasts was assessed by using mineralising surface as a proportion of osteoid surface (sLS+dLS/OS); and the efficiency of active osteoblasts was assessed using the ratio of double to total labelled surface (dLS:tLS). The values of each parameter were standardised using age and sex matched control data and a three dimensional matrix was used to identify groups of patients with similar patterns of altered function.**

***Results*—The largest group (60 cases) showed a reduction in all three parameters, while a small group (9 cases) had normal osteoblast function. However, one group showed reduction in osteoblast number only (23 cases), while another group showed a normal number of osteoblasts but both reduced percentage and efficiency of activity (14 cases). The results also suggest that efficiency of activity falls first and that this eventually leads to exit from the active pool.**

***Conclusions*—These results demonstrate the presence of heterogeneity of osteoblast dysfuction in osteoporosis, indicating that the disease is caused by interference at a variety of target sites along the pathway of osteoblast proliferation, differentiation, and activation. Greater understanding of this pathway and of the variety of alterations in the pathway that can occur in osteoporosis may allow more focused therapy for different patient groups identified on the basis of histomorphometric analysis.**

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Osteoporosis is a clinical syndrome characterised by a reduction in bone mass (osteopenia), usually in the trabecular compartment, and resultant low trauma fractures.[1] It is an important cause of morbidity and mortality in the elderly, especially in women, and the economic cost of osteoporotic femoral fractures alone is substantial. At the cellular level it has been shown to be caused by an imbalance in osteoblast bone formation and osteoclast bone resorption,[2] different pathogenic mechanisms being responsible for alteration in each side of the equation. Treatment for established osteoporosis aims to increase bone mass by either reducing ostoclastic activity, or increasing osteoblastic bone formation, or both.[1] Unfortunately, standard treatment regimens are unsuccessful in restoring bone mass.[3] Various studies are beginning to demonstrate that osteoporosis is a heterogeneous condition.[4–9] Clinical studies have sought to explain this in terms of groupings defined on the basis of age and other clinical characteristics, but these have not proved useful.[1 10–12] Most histomorphometric studies have concentrated on static measurements, and only a few have attempted to analyse osteoblast function. In a recent study using multifactorial analysis of histomorphometric data from postmenopausal patients we demonstrated the presence of clusters of patients characterised by differences in osteoblastic and osteoclastic function.[13] From that study it became clear that there was an urgent need to investigate in greater depth the functional changes in osteoblastic activity in osteoporosis. Theoretically, these could be due to a reduction in osteoblastic number, in the percentage of osteoblasts active at any one time, and/or the efficiency of those active cells. Insight into differences in each of these factors in different patients could have therapeutic implications, particularly as new understandings of osteoblast control mechanisms lead to new treatment regimens.[3] To investigate osteoblast function we used histomorphometric data to provide measures of osteoblasts in 153 patients with established osteoporosis.

Methods

Histomorphometric data from iliac crest biopsies were abstracted from the files of 153 patients with established osteoporosis referred to either Hope Hospital, Salford or the Manchester Royal Infirmary, Manchester. The diagnosis of established osteoporosis was determined radiologically by the presence of at least one non-traumatic vertebral crush fracture or a lumbar spine (L2–L4) bone mineral density less than two standard deviations below peak bone mass (assessed by dual x ray absorptiometry). The biopsies were fixed in absolute

Table 1 Mean values for both raw data and normal population values for each paramater, together with spread of normal means and standard deviations

	Osteoblast number (ObS:BS)	*% active (sLs + dLs/OS)*	*Efficiency of active osteoblasts (dLs:tLs)*
Mean normal value	5.0	71.2	55.1
Mean raw value	3.0	49.9	26.7
Spread of normal mean	4.2–6	48.3–81	43.3–58
Spread of normal SD	0.5–1. 05	5.4–7.55	3.7–5.55

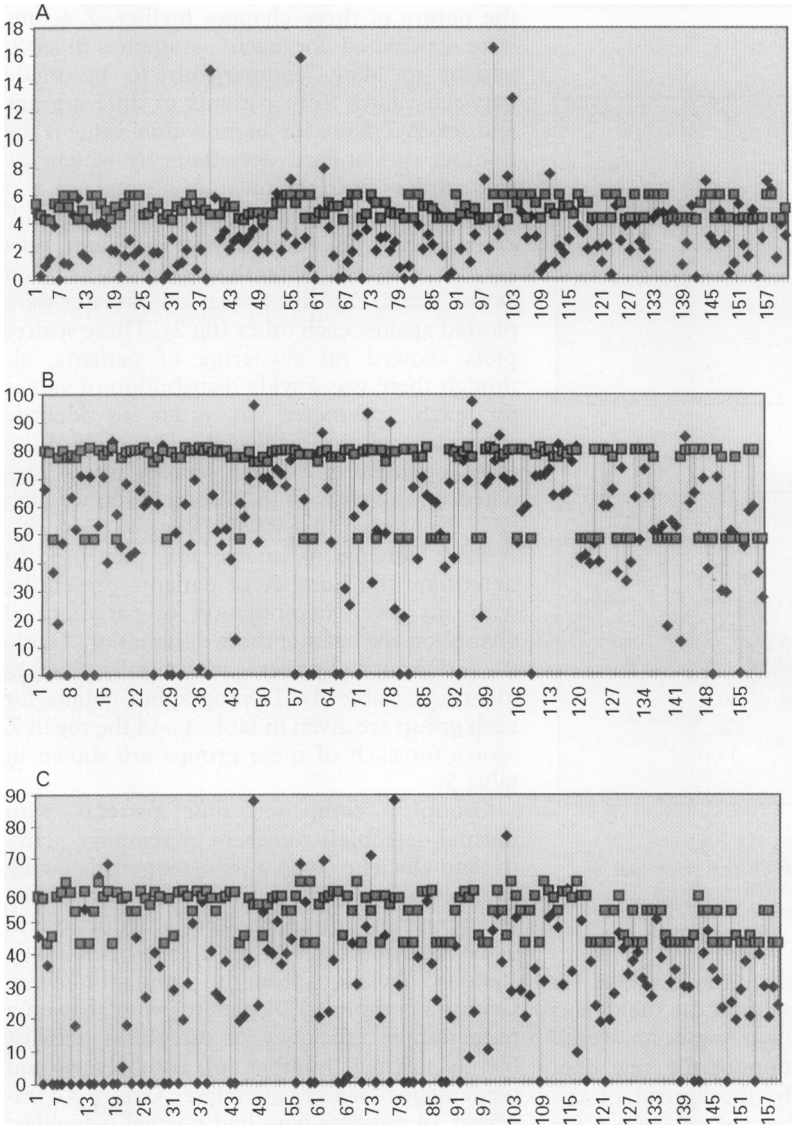

Figure 1 Graph of raw value (shaded square) and corresponding age/sex matched mean (black diamond) for each patient in parameter: (A) osteoblast number (ObS:BS); (B) percentage of active osteoblasts (sLS + dLS/OS); (C) efficiency of osteoblasts active (dLS:tLS).

Table 2 Three dimensional matrix of parametrical changes based on Z scores

NNN	9	HNN	1	LNN	12
NNH	3	HNH	2	LNH	1
NNL	14	HNL	2	LNL	23
NHN		HHN		LHN	
NHH	1	HHH		LHH	1
NHL	2	HHL		LHL	1
NLN	2	HLN		LLN	3
NLH		HLH	1	LLH	
NLL	14	HLL	1	LLL	60

Each cell is given in the format: osteoblast number (ObS:BS); percentage of osteoblasts active (sLS + dLS/OS); efficiency of osteoblasts active (dLS:tLS), each parameter being designated either normal (N), high (H), or low (L). The number of cases with each combination of changes is indicated.

alcohol and processed three times in LR white resin monomer (London Resin Co, Basingstoke, UK), the last two taking place under reduced pressure. Resin polymerisation was carried out overnight at 60°C. Twenty seven 5 μm step serial sections were cut through each block with a tungsten tipped knife on an LKB powered microtome. Groups of three sections were stained with toludine blue (pH 4.2), or using the modified Giemsa or Von Kossa techniques. For fluorescence microscopy, 20 μm unstained sections were cut at different levels throughout the block. Histomorphometric analysis was carried out manually by an independent observer unconnected with the subsequent analysis. Only biopsies with an adequate core of bone, including both trabecular and cortical bone, were included. Each patient had received two doses of oral dimethyl-chlortetracycline (10–15 mg/kg body weight), the first 15–18 days before the biopsy, and the second 10 days later. Biopsy was performed within four to seven days of the last tetracycline dose.

When tetracycline labelled sections were analysed, the total length of mineralising surface (tLS) was divided into two components—those with two (double labelled surface, dLS) and those with only one label (single labelled surface, sLS). The latter represented regions of the bone surface that had either stopped or started mineralisation between the two doses of label. Osteoid surface was identified in toludine blue stained sections and defined as an unmineralised surface at least 3 μm thick. Parameters were measured using standard techniques[14–16] and defined according to the terminology proposed by Parfitt *et al.*[17]

Counting osteoblasts is often impractical because of their number and indistinct cell boundaries. A surrogate for osteoblast number was assessed by measuring the ratio of osteoblast surface to bone surface (ObS:BS). Two measures of mineralising surface were used. The proportion of osteoid surface bearing either single or double label (sLS+dLS/OS) was used to assess the percentage of osteoblasts actively mineralising at any one time, while the ratio of double to total labelled surface (dLS:tLS) was used to indicate the degree to which those active at any one time remained active thereafter (the efficiency); if dLS was equal to tLS (dLS:/tLS = 100%) then all those active when the first label was applied would also have been active when the second label was applied and, therefore, could be said to be functioning at a high degree of efficiency.

Results

Each of the three parameters in each patient was expressed as raw values and as Z scores. The mean and the spread of the raw values for each of the three parameters are shown in table 1, together with the mean and spread of the population means against which the raw values were compared to obtain Z scores. The population mean and standard deviation varied with age and sex within each parameter and so, although the appropriate age/sex matched

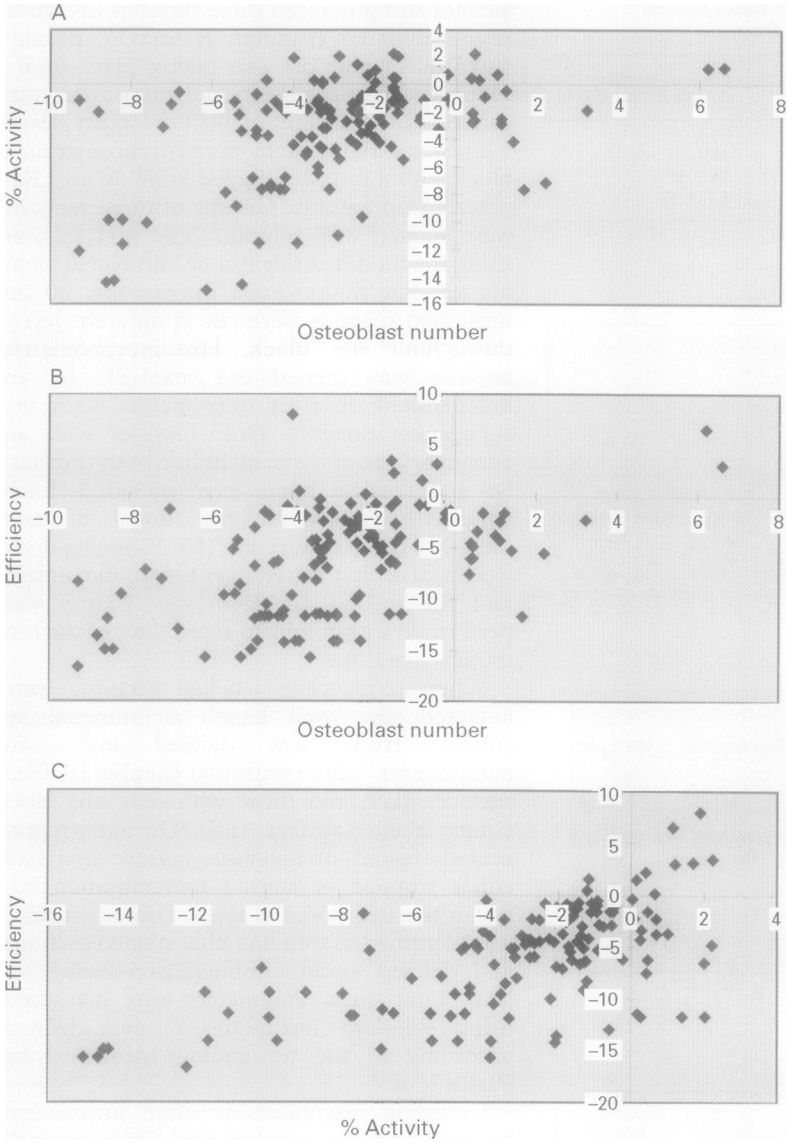

Figure 2 Scatter plots of Z scores of (A) osteoblast number (ObS:BS) v percentage of active osteoblasts (sLS + dLS/OS); (B) osteoblast number (ObS:BS) v efficiency of osteoblasts active (dLS:tLS); (C) percentage of active osteoblasts (sLS + dLS/OS) v efficiency of active osteoblasts (dLS:tLS).

means were used to produce the Z scores for each parameter in each patient, the mean and spread of the means is shown to give an overall impression of the deviation of the raw data from the norm. Similarly, the spread of the standard deviations of the normal values for each parameter is given in table 1. The results demonstrate a marked reduction in the mean raw value compared to the mean normal population value for each parameter. The raw values and the corresponding age/sex matched normal population means, which therefore vary from patient to patient, for the entire dataset of each parameter are shown in fig 1. To analyse the nature of these changes further, Z scores were calculated for each parameter in each patient to allow comparisons to be made between values from patients of differing age and sex. A Z score for an individual value is the number of standard deviations by which the value differs from the mean of normal age and sex matched controls for the local population.[18] Z scores allow the standardisation of data and a Z score of either greater than 2 or −2 was taken as abnormal. Z scores for each parameter were plotted against each other (fig 2). These scatter plots showed no clustering of patients, although there was a wide distribution of values for each parameter. In order to identify subgroups within this distribution, each of the three parameters in each patient was designated on the basis of its Z score as either high ($Z > 2$), normal ($2 > Z > -2$) or low ($Z < -2$). A three dimensional matrix was then used to determine the number of patients presenting with any given combination of parametrical change on the basis of these designations (table 2), and groups of patients with similar changes identified (table 3). Demographic details for each group are given in table 4 and the mean Z scores for each of these groups are shown in table 5.

Group 1 comprised nine patients with normal osteoblast numbers, percentage activity, and efficiency who were osteoporotic owing to excess osteoclastic activity in six cases, while the remaining three showed a reduction in osteoblast number that did not fall below two standard deviations from the population mean. Group 2 comprised 14 patients who showed a reduction in efficiency of osteoblast activity, but a normal number of osteoblasts and percentage of active osteoblasts. Group 3 comprised 14 patients who had normal osteoblast numbers, but showed a reduction in both the percentage and efficiency of osteoblasts active. Group 4 comprised 23 patients who showed a normal percentage of active osteoblasts, but a reduction in absolute osteoblast number and the efficiency of active osteoblasts. Group 5 comprised 12 patients who had low numbers of osteoblasts, but a normal percentage and

Table 3 Groups of patients with each combination of parametrical change

	No.	*Osteoblast No (ObS:BS)*	*% active (sLs + dLs/OS)*	*Efficiency of active osteoblasts (dLs:tLs)*
Group 1	9	Normal	Normal	Normal
Group 2	14	Normal	Normal	Low
Group 3	14	Normal	Low	Low
Group 4	23	Low	Normal	Low
Group 5	12	Low	Normal	Normal
Group 6	60	Low	Low	Low
Others*	21	Aggregate of smaller groups and not analysed further		

*Combinations represented in fewer than 4 patients (21 cases in total) were not considered large enough for independent analysis.

Table 4 Demographic details of patients in the different histomorphometric groups

	Males:females	*Mean age (SD)*
Group 1	1:8	59.5 (10.5)
Group 2	2:12	60.1 (8.1)
Group 3	2:12	56.1 (7.2)
Group 4	5:18	56.5 (8.6)
Group 5	4:8	53.4 (11.0)
Group 6	26:34	52.7 (12.0)

Table 5 Z scores of histomorphometric parameters in the different groups

	Osteoblast No (ObS:BS)	*% active (sLs + dLs/OS)*	*Efficiency of active osteoblasts (dLs:tLs)*
Group 1	−0.5	−1.0	−0.9
Group 2	−0.8	−0.5	−4.8
Group 3	−0.2	−3.9	−6.1
Group 4	−3.5	−0.9	−5.5
Group 5	−3.7	−1.0	−1.0
Group 6	−4.4	−6.5	−10.4

efficiency of osteoblast activity. Group 6 comprised 60 patients who showed a reduction in all three parameters. In addition 21 patients showed a variety of other combinations of parametrical change, but none of these smaller groupings contained more than three patients and they were not considered large enough for meaningful conclusions to be drawn from them. Although there were differences in the proportion of male and female cases and mean ages of cases between groups, these were not statistically significant.

Discussion

Osteoporosis is a clinical syndrome characterised by the end point of osteopenia and resultant low trauma fractures. It encompasses a widely heterogeneous population and previous studies have indicated that a better understanding of the mechanisms underlying osteoporosis and a firmer basis for therapeutic intervention could be achieved by subclassification to produce more homogeneous patient groups.[19] Most attempts have utilised clinical parameters, but recently a histomorphometric approach has been used that demonstrates different groups with altered osteoblast and osteoclast function. This emphasises the need to know and target the cellular mechanisms leading to reduction in bone mass[3 20-22] and directs attention to the different patterns of cell dysfunction that can occur in the disease. Demonstrating reduced osteoblast activity and/or number could be of considerable pertinence, especially in view of the present intense research into the processes modulating osteoblast proliferation and activation.[23-28] We analysed histomorphometric data from 153 patients with established osteoporosis in order to investigate and characterise the existence of different patterns of osteoblast dysfunction.

A small number of patients (group 1) showed either no significant change in any of the osteoblast parameters, or an increase in osteoclastic activity. The largest single group (group 6) showed a decrease in all three parameters, that is, reduced osteoblast number, reduced percentage of active osteoblasts, and reduced efficiency of active cells, indicating poor recruitment of osteoblasts from the precursor pool and poor activity once recruited. However, the majority of patients belonged to neither group but showed various deficiencies in recruitment and/or activity. Both osteoblast number and function were reduced in 83 patients. These could be subdivided into two subgroups (groups 4 and 6), the majority (group 6) showing reduction in both functional measures (percentage activity and efficiency of activity), while those in group 4 showed a fall in efficiency only, a fall in the efficiency of active cells occurring more frequently than a fall in the percentage of cells active. This suggests that the more common alteration in function is a relative one, reflected in a fall in the level of efficiency of active osteoblasts, rather than an absolute one of a reduction in the number of active osteoblasts. In contrast, there were no large subgroups with normal efficiency but reduced percentage of active osteoblasts. This emphasises that a relative reduction in osteoblast activity is more common than an absolute one and suggests a sequential change with an initial relative reduction in level of activity which eventually leads to a total lack of function and, therefore, to a reduction in the percentage of active osteoblasts. In support of this, a reduced efficiency was observed in a total of 101 cases and was the most frequent abnormality detected, and although the number of cases showing reduced osteoblast number was similar (95 cases), the group showing reduction in osteoblast number only was the smallest (12 cases), further indicating that reduced activity is more frequent than absolute number. The mechanisms involved in control of osteoblast number and function do seem to be separate in some patients, with groups 2 and 3 showing normal numbers of osteoblasts but reduction in one or both of the functional parameters. Similarly, group 5 showed a reduction in osteoblast number alone. In 21 patients the groupings involved very small numbers of patients (up to three), and although this reflects further the heterogeneous nature of osteoporosis and supports the approach used, these groups were omitted from further analysis since while they may represent less frequent patterns of dysfunction, the possibility that they represent outliers cannot be excluded.

Previous studies have used cluster analysis to identify discrete groups.[13] Although we have identified broad groups, scatter plots of the data showed that no formal clusters representing discrete entities were present, but rather that the data were normally distributed over a wide area.[29] However, even when discrete clusters or entities do not exist in a dataset there is still a clinical and categorical utility in splitting or subdividing the data using set limits, such as the upper and lower limits of the normal distribution, as in this case. This allows datasets which necessarily lie along a spectrum, such as those for many diseases, to be subdivided and simplified in a meaningful manner. We have analysed histomorphometric data from a large cohort of osteoporotic patients to determine the nature of changes in osteoblastic function and have demonstrated that there are different patterns of osteoblastic function. In particular, the results suggest that the number and function of osteoblasts in osteoporosis may be altered in different ways, although in every case the end point is a reduction in bone mass. Our data could be interpreted as showing that a reduction in cellular function is caused initially by a reduction in the level of activity, which leads eventually to cessation of activity and consequent exit from the active pool. These results emphasise that at the time of presentation the cellular dysfunction is heterogeneous. To reverse the dysfunction to improve bone mass necessitates recognising the specific defect in a given patient at the time of presentation and emphasises the utility of histomorphometry in this analytical process. In addition, these results indicate the nature of the possible cellular mechanisms operating, and demonstrate that more detailed understanding

of the cellular pathology of osteoblasts in osteoporosis will allow more focused and effective treatment. In particular, our data indicate that a significant proportion of patients have decreased numbers of osteoblasts. To target this defect would require a greater understanding of the site and differentiation pathways of pre-osteoblasts than is available currently.

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