Morphometry of bone

Morphometric methods are commonly used in the diagnosis of diseases of bone. These may be divided into static and dynamic measurements. Static measurements include: (i) the proportion of trabecular surface which is resting, resorbing or covered by osteoid (by a perimeter intersect technique); (ii) the thickness of osteoid seams; and (iii) the proportion of the section occupied by mineralized bone, osteoid, woven bone, lamellar bone or fibrous tissue (by a point-counting technique or by computerized image analysis). Dynamic studies may be performed using a tetracycline labelling method. When a single dose of tetracycline is administered, it becomes incorporated at the mineralization front; this can be visualized as a single line in undecalcified sections examined under ultraviolet light. By giving two doses of tetracycline at a known interval and measuring the distance between the two lines of incorporation, it is possible to measure the mean rate of mineralization.

Osteoporosis

Osteoporosis is defined as a decreased amount of bone per unit volume. There is no decrease in the external dimensions of the bone, which is histologically normal, but there is a reduction in the amount of trabecular bone per unit volume of cancellous bone and there may also be thinning of the cortex (Fig. 11.1). Fragility of the bone may lead to spicules being fractured during the biopsy procedure [4]. Histomorphometry shows that approximately 60% of patients have reduced numbers of osteoblasts [5]. The disorder is common in the elderly, in whom it causes considerable morbidity as a result of increased susceptibility to fractures. Osteoporosis is
more common in women and its frequency increases progressively after the menopause; severe osteoporosis has also been reported in men [6] and in children [7]. The cause is not known but genetic factors have been implicated [8]. The mechanism is thought to be increased osteoclastic resorption in conjunction with a reduced rate of bone formation [9]. In a minority of cases, osteoporosis is secondary to other diseases such as Cushing’s syndrome, thyrotoxicosis, hypopituitarism, malnutrition, malabsorption and chronic heparin or corticosteroid administration. Diffuse osteoporosis is also sometimes associated with multiple myeloma, aplastic anaemia, chronic granulocytic leukaemia, systemic mastocytosis and polycythaemia rubra vera. Localized osteoporosis can occur following immobilization of a limb.

Plain radiographs of the vertebral column are usually only abnormal in advanced disease and are an unreliable means of diagnosing osteoporosis. An assessment of the severity of osteoporosis can be made using biopsies from the iliac crest [10]. The trabeculae are thinned and are reduced to slender strands, often with complete transection, but they are otherwise normal and there is no increase in the width of osteoid seams. Rather, osteoid seams and the number of osteoblasts tend to be reduced. Accurate assessment of the severity of osteoporosis requires the use of static morphometric measurements. Iliac trabecular bone normally occupies approximately 23% (SD ± 3%) of the measured area in adults under 50 years of age, but this falls to 16% (SD ± 6%) in elderly individuals [11]. When the amount of trabecular bone falls below 11% (SD ± 3%), vertebral fractures tend to occur [3].

Recently, reliable non-invasive techniques for the measurement of bone mass at the sites most prone to fracture have become available; these include dual proton absorptiometry, quantitative computerized tomography (CT) and dual-energy X-ray absorptiometry [12]. It seems likely that these techniques will render iliac crest biopsy unnecessary for the diagnosis of osteoporosis.

The peripheral blood is normal in osteoporosis; the bone marrow is essentially normal, although increased numbers of mast cells have been reported [13]. There may, however, be an appearance of hypocellularity since the loss of bone leads to an increased percentage of the marrow cavity being occupied by fat cells.

**Osteomalacia**

Osteomalacia literally means softening of the bones. It is a consequence of failure of mineralization of bone matrix, resulting in abnormally wide osteoid seams around the bone trabeculae (Fig. 11.2). Numerous causes of osteomalacia have been described but the majority of cases result from deficiency of vitamin D due, in turn, to: (i) reduced
intake; (ii) inadequate exposure to sunlight; or (iii) abnormalities of absorption or metabolism of the vitamin (as in renal disease). Rarely, osteomalacia is caused by a hereditary end-organ resistance to vitamin D and its metabolites. In adults, severe osteomalacia predisposes to fractures. In children, in whom the epiphyses have not yet closed, the clinical picture is that of rickets, with its characteristic skeletal deformities.

In normal adults, approximately 0.5% of the whole bone area (that is, bone plus marrow) is made up of osteoid, which covers 13% (SD ± 7%) of the trabecular bone surface. A mineralization front is seen in more than 60% of the surface osteoid. Under polarized light, normal osteoid seams are seen to be composed of between one and four lamellae [3]. In osteomalacia there is an increase in both total osteoid and the area of trabecular surface covered by osteoid; the osteoid seams are greater than five lamellae in thickness and the mineralization front is decreased. Double tetracycline labelling shows a reduction in the mineralization rate (normal mean value 0.7 µm per day). The bone marrow is usually normal.

The peripheral blood and bone marrow aspirate are usually normal in osteomalacia. However, children with severe vitamin D deficiency have been reported to develop a hypocellular bone marrow with fibrosis, thrombocytopenia and a leuco-erythroblastic anaemia associated with extramedullary erythropoiesis [14].

Hyperparathyroidism

Skeletal changes occur in both primary and secondary hyperparathyroidism [15–18]. The extent of these changes depends on the severity and duration of the underlying disease. Primary hyperparathyroidism is usually the result of a parathyroid adenoma; primary hyperplasia is a less common cause. Very rarely, there is an underlying parathyroid carcinoma. Secondary hyperparathyroidism is usually a consequence of renal disease; less commonly the underlying cause is intestinal malabsorption and rare cases have been reported following gastric bypass surgery for treatment of severe obesity [19]. In one reported case, high levels of secretion of parathyroid hormone-related protein, by cells of an HTLV-I-positive adult T-cell leukaemia, produced bone disease indistinguishable from osteitis fibrosa cystica [20]. Parathyroid hormone and related molecules increase osteoclast generation and function, resulting in increased bone resorption; more recently, parathyroid hormone has also been shown to increase bone formation [21].

Skeletal changes in hyperparathyroidism follow a predictable sequence. The earliest change is the presence of excess osteoid seams around the bone trabeculae, an appearance that closely resembles osteomalacia. Later, osteoclasts are activated and there is increased bone resorption. Howship’s lacunae and osteoclasts are prominent and there is fibrosis of the paratrabecular marrow (Fig. 11.3).
This appearance is known as osteitis fibrosa. The Howship’s lacunae may be filled by large osteoclasts and, as the lacunae enlarge, trabeculae may be transected. Fibrosis increases and fibrous tissue eventually fills some intertrabecular spaces completely. There is a moderate increase in the vascularity of the marrow. At this stage, macroscopic cysts may be visible. Haemosiderin-laden macrophages are frequently seen within the fibrous tissue, resulting from microhaemorrhages; foreign body-type giant cells may also be present. This final stage is sometimes referred to as osteitis fibrosa cystica.

Only a minority of patients with hyperparathyroidism have significant bone disease and, with earlier diagnosis and treatment, severe manifestations (osteitis fibrosa cystica) are rarely seen nowadays. The features are important to remember, however, since bone marrow biopsy is occasionally performed to investigate either hypercalcaemia or radiographic lesions suspicious of metastatic carcinoma in patients with unsuspected severe hyperparathyroidism [22–24].

There are no specific peripheral blood or bone marrow aspirate abnormalities associated with primary hyperparathyroidism although mild anaemia may occur [25].

**Renal osteodystrophy**

The majority of patients with chronic renal failure have some abnormality of bone structure [17,18]. The manifestations are complex [26] and include combinations of bone disease due to secondary hyperparathyroidism (80–90% of cases), osteomalacia (20–40% of cases) and osteosclerosis (around 30% of cases) [3,27]. The most severe changes are seen in those patients with chronic renal failure who are maintained on dialysis. There is marked geographical variation in the nature of renal osteodystrophy, with hyperparathyroid bone disease predominating in the United States and osteomalacia in the United Kingdom. In adults, the symptoms are rarely severe. Secondary hyperparathyroidism in renal failure is consequent on hypocalcaemia which is, in turn, caused by a combination of reduced hydroxylation of vitamin D and phosphate retention by the kidney [28]. The major cause of renal osteomalacia is the toxic action of aluminium derived from dialysate; geographical variations in the incidence are related to the concentration of aluminium in water used for dialysis [28,29]. The use of de-ionized water for dialysis has resulted in a fall in the incidence of renal osteomalacia in some centres [3].

The histological changes are identical to those previously described in hyperparathyroidism (osteitis fibrosa), often combined with those of osteomalacia (Fig. 11.4). Bone trabeculae may have tunnels excavated within them by osteoclasts (Fig. 11.5) but the most severe changes of osteitis fibrosa cystica are seen only rarely. Osteosclerosis, due to increased formation of woven bone, may be widespread...
throughout the skeleton. With advanced renal osteodystrophy, the bone marrow may be hypocellular and extensively fibrosed with proliferation of vessels, particularly arterioles. Patients with renal osteodystrophy have been noted to have mononuclear cells within the haemopoietic marrow which are positive for tartrate-resistant acid phosphatase; these cells are probably osteoclast precursors [30,31].

There may also be abnormal deposition of aluminium or iron. Aluminium deposition occurs at the junction between osteoid and mineralized bone. It is detected as a red/purple line in an Irwin stain using an undecalcified biopsy [32] and provides evidence of exposure to an excessive aluminium concentration in the dialysate. Aluminium may also be detected inside bone marrow cells, possibly macrophages [33]. In dialysis patients who are iron-overloaded, iron may also be deposited at the mineralization front [34]; iron deposition may be aetiologically related to osteomalacia.

Renal osteodystrophy may contribute to the anaemia of chronic renal failure and may also cause leucopenia or thrombocytopenia [35]. There are no
specific associated morphological abnormalities in the peripheral blood or bone marrow aspirate although a ‘dry tap’ may occur. Response to erythropoietin therapy is worse in those patients who have more severe secondary hyperparathyroidism [36] and iron overload [37].

**Paget’s disease of bone**

Paget’s disease of bone is a disease of unknown etiology, characterized by increased osteoclastic resorption of bone followed by unco-ordinated formation of disordered reactive bone. Infection by a virus of the paramyxovirus group (including measles virus, respiratory syncytial virus and canine distemper virus) has been investigated extensively as a possible cause but the findings have been inconclusive [38,39]. In one series of patients, molecular genetic analysis has failed to find evidence of paramyxovirus RNA sequences in tissue from pagetic bone [40]. Paget’s disease of bone occurs with familial clustering in some instances [39,41] and genetic linkage to chromosome 18q21–22 has been established in some, but not all, families [41–43]. Occupational exposure to lead has also been proposed as a possible contributory factor in development of the disease [44,45]. Paget’s disease is uncommon before the age of 40 years and becomes progressively more common with increasing age. In approximately 15% of cases, the disease is confined to a single bone (monostotic). In the majority of cases, several bones are involved, most commonly the vertebral column, pelvis, femur, skull and sacrum. The clinical features are pain, due to microfractures, and neurological symptoms consequent on damage to nerves as they pass through the foramina of the skull and vertebrae. Rarely, there is high-output cardiac failure as a result of the highly vascular lesions acting as arteriovenous shunts. The development of osteosarcoma is an uncommon, but well-established, complication of Paget’s disease.

In the initial stages of the disease, increased bone resorption is the dominant feature. Trabeculae have a scalloped appearance due to increased numbers of resorption bays containing very large osteoclasts with numerous nuclei (Fig. 11.6). The increased resorption of bone is followed by deposition of disordered woven bone. Osteoblasts are increased. At this stage, the marrow cavity is partly occupied by loose connective tissue; there is increased vascularity with arterioles and capillaries being particularly increased. Eventually, new bone formation becomes the dominant feature and lamellar bone is laid down causing thickening of the bone trabeculae. However, the lamellar bone is laid down in an unco-ordinated and haphazard fashion. The irregular cement lines, which appear more basophilic

**Fig. 11.6** BM trephine biopsy section, Paget’s disease of bone, showing thickening of bone trabeculae, numerous resorption bays (Howship’s lacunae) containing large osteoclasts and replacement of marrow by vascular connective tissue. Paraffin-embedded, H&E $\times 39$. 
than the surrounding bone, form a characteristic mosaic or tessellated ('tile-like') pattern that is a hallmark of Paget’s disease (Fig. 11.7). Each of the cement lines represents a surface where bone resorption has been followed by bone deposition. The trabeculae eventually become massively thickened and encroach upon the marrow cavity.

Severe Paget’s disease may have an associated mild anaemia. The bone marrow aspirate does not show any specific abnormality but increased osteoblasts and osteoclasts are sometimes seen.

It should be noted that prolonged bleeding, consequent on the greatly increased vascularity, has been reported following trephine biopsy in a patient with Paget’s disease [46].

**Osteosclerosis**

Osteosclerosis is the term used to describe a group of conditions in which there is an increase in the amount of bone per unit volume, usually resulting from increased bone formation. Osteosclerosis is most often seen in conjunction with severe bone marrow fibrosis, either in a myeloproliferative disorder or in metastatic carcinoma. It has been reported in patients with systemic mastocytosis [47] although the more usual finding in this disease is diffuse osteoporosis. Osteosclerosis occasionally occurs in multiple myeloma but osteolytic lesions are much more characteristic. It is also associated with plasma cell neoplasia in the polyneuropathy, organomegaly, endocrinopathy, M-protein, skin changes (POEMS) syndrome (see page 356) and has been reported in patients with hairy cell leukaemia, in whom it has regressed or stabilized with treatment of the underlying lymphoproliferative disease [48,49]. Osteosclerosis may occur without primary bone marrow disease in the congenital condition designated osteopetrosis (see below) and also, rarely, in adults in the absence of any associated disease (Fig. 11.8). The cause of isolated osteosclerosis in adults is unknown; some reported cases have suggested an association with intravenous drug abuse [50].

In the myeloproliferative disorders (see Fig. 5.21), the term osteomyelosclerosis [51,52] is sometimes used. The new bone may be either bone formed on the endosteal surface of trabeculae, leading to marked trabecular thickening or, less commonly, irregular spicules of metaplastic woven bone within the fibrous tissue. The strands of woven bone may form an irregular network in the intertrabecular spaces and, in severe cases, the medullary cavity is almost completely obliterated. Some conversion of woven bone to mature bone occurs.

Metastases from various types of carcinoma may cause dense bone marrow fibrosis and osteosclerosis but these changes are most commonly associated
DISEASES OF BONE

Thyroid disease [53,54]

Thyrotoxicosis has been found to be associated with osteoporosis, an increased percentage of osteoid and a marked increase in osteoclasts. Hypothyroidism is associated with osteosclerosis, normal or decreased osteoid percentage and reduced osteoclasts.

Bone necrosis and repair

Conditions causing bone marrow necrosis (see page 128) also cause necrosis of trabecular bone in many instances. In the acute phase, necrotic bone is recognized by the absence of osteocytes from lacunae. It should be noted that occasional lacunae may appear empty in normal bone if the plane of section does not pass through an osteocyte nucleus.

Repair occurs by appositional new bone formation. Woven bone is deposited on the surface of the dead lamellar bone (Fig. 11.9). This is followed by the normal processes of bone remodelling in which the woven bone is replaced by lamellar bone.

Osteopetrosis (Albers-Schoenberg disease)

Osteopetrosis, also known as marble bone disease or Albers-Schoenberg disease, is a hereditary metabolic disease consequent on a defect in osteoclast function [55,56]. Osteoclasts may be increased (Fig. 11.10), decreased (Fig. 11.11) or present in normal numbers but are always qualitatively abnormal [57,58]. The result is osteosclerosis with gradual obliteration of the marrow cavity by both bony encroachment and associated fibrosis. Although the bone density is increased, the bone is more fragile than normal. Osteopetrosis occurs in an autosomal recessive form, which manifests itself...
Fig. 11.9 BM trephine biopsy section. Newly deposited woven bone on the surface of a necrotic trabecula. Paraffin-embedded, H&E ×188.

Fig. 11.10 BM trephine biopsy section from a child with osteopetrosis, showing marked increase in osteoclast numbers and bone marrow fibrosis. H&E ×188. (By courtesy of Adrienne Flanagan, London.)

Fig. 11.11 BM trephine biopsy section from a child with osteopetrosis, showing abnormal bone structure and no detectable osteoclasts. H&E ×188. (By courtesy of Adrienne Flanagan, London.)
Osteopetrosis

Osteopetrosis is a group of diseases characterized by bone thickening due to defective osteoclastic activity. In the severe infantile form, there is progressive bone thickening leading to complications such as leuco-erythroblastic anaemia and thrombocytopenia. In the milder adult form, anaemia is usually mild. Osteopetrosis is often associated with other features such as blue sclerae, laxity of joints, and abnormalities of dentition.

Osteogenesis imperfecta

Osteogenesis imperfecta is a group of related hereditary diseases characterized by bone fragility and deformities. The most severe variant (type II) is autosomal recessive and is fatal in the perinatal period. Other variants have less severe manifestations and may be compatible with survival into adult life. Histologically, thinning of the cortex and trabeculae is common.

Problems and pitfalls

Handling bone biopsy cores can lead to artefacts that mimic other conditions. For example, damage during biopsy core preparation may lead to bone fragments that resemble Paget's disease or bone necrosis. Incomplete decalcification can result in basophilic staining of bone with haematoxylin and eosin, which may mimic abnormal bone growth. Careful examination of sections from multiple levels can help distinguish these artefacts.

Reactivities of some monoclonal antibodies with normal bone components should not be mistaken for reactions with abnormal cells. For example, VS38c and CD30 react with osteoblasts, while antibodies of the CD68 cluster react with osteoclasts.

Incomplete decalcification may lead to basophilic staining of bone with haematoxylin and eosin, particularly affecting central areas within trabeculae, which may suggest abnormal bone growth. If sections are examined from several levels through the biopsy specimen, it is usually clear that the incomplete decalcification is more extensive towards the centre of the core. When the sections are studied carefully, a normal pattern of lamellae and lacunae can be seen, even in partly calcified areas. If poor decalcification causes difficulty with cutting or staining of sections, surface decalcification of the wax block can be used but the routine laboratory protocol for decalcification should also be reviewed.

Trabeculae of bone may become detached from trephine biopsy sections during staining, and spaces remaining may mimic dilated sinusoids. Careful attention to their contours and comparison with preserved trabeculae usually makes their true nature obvious.

Regenerating bone post-chemotherapy may mimic hyperparathyroid bone disease or renal osteodystrophy but does not show the tunnelling of trabeculae by fibrous tissue that characterizes these conditions.

Newly made woven bone may have large lacunae, and the resident osteocytes may not be apparent in a particular section. The bone may therefore appear to lack osteocytes and may be mistaken for necrotic bone. However, only a few lacunae will appear acellular, and the lack of lamellar structure (confirmed, if necessary, with a reticulin or trichrome stain) will confirm that woven bone is present.

The irregular bone of severe Paget’s disease may mimic osteomyelosclerosis due to idiopathic myelofibrosis or metastatic malignancy. The diagnosis of Paget’s disease is confirmed by the presence of giant osteoclasts and the distinctive tessellated pattern of irregular bony plates with scalloped edges that make up individual trabeculae.

Reactivities of some monoclonal antibodies with normal bone components should not be mistaken for reactions with abnormal cells. For example, VS38c and CD30 react with osteoblasts, while antibodies of the CD68 cluster...
react with osteoclasts, the latter being of macrophage origin.

References

containing high and low concentrations of aluminium. 


