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Original Paper

Monitoring of Bone Metastases

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INTRODUCTION

A VARIETY of treatments, including radiotherapy, endocrine treatment, chemotherapy and bisphosphonates are used for the treatment of metastatic bone disease and evaluation of their effects is important for both routine clinical practice and research. The current imaging methods used to assess response to these treatments are qualitative and routinely include plain radiographs, radionuclide bone scans and, in particular situations, computerised tomography (CT). The response assessment of bone metastases to therapy is notoriously difficult; the events in the healing process are slow to evolve and quite subtle, with sclerosis of lytic lesions only beginning to appear 3–6 months after the start of therapy [1]. Bone is the only site of metastatic disease that has separate criteria for evaluation of response to treatment, based on bone repair and destruction rather than changes in tumour volume [2]. A complete review of the bone radiographs since the start of a treatment is necessary to evaluate response, a slow and tedious process.

Assessing response to treatment in bone is more difficult than evaluation of disease in viscera and soft tissues where tumour measurements can usually be taken. This results in reports of lower response frequencies to systemic treatments in the skeleton compared with other sites of disease. Complete response in non-osseus sites affected by breast cancer occurs in 10–20% of patients, but a complete response (CR) in bone with return of normal trabecular pattern, or resolution of sclerotic metastases is very rare. Although this could represent some biological phenomenon of site-specific resistance, this is unlikely and the discrepancy in response frequency is almost certainly a reflection of the insensitivity of the assessment methods. Consequently, patients with metastatic disease confined to the bone are frequently excluded from many therapeutic trials and patients with widespread metastatic disease (including bone) rely on the soft tissue or visceral disease as markers of response.

While recognising that the changes seen on serial radiographs remain the 'gold standard' for evaluating response to therapy, new methods of assessing response are needed, both to improve patient management and to evaluate specific treatments. A number of alternatives or adjuncts to assessment based on plain radiographs have been suggested. None are ideal, each having advantages and disadvantages as outlined below. However, as will be shown later, developments in biochemical evaluation of bone metabolism offer the possibility of real progress in this area.

IMAGING OF BONE METASTASES

Plain radiology

A skeletal radiograph indicates the net result of bone resorption and repair. For a destructive lesion in trabecular bone to be recognised on a plain radiograph, it must be >1 cm in diameter, with loss of approximately 50% of the bone mineral content [3]. It is this predominance of lysis or sclerosis which gives rise to the characteristic radiographic appearance of a bone metastasis. When bone resorption predominates, focal bone destruction occurs and the lesion has a lytic appearance. Conversely, in bone metastases characterised by increased osteoblast activity, the lesion appears sclerotic. Even when one element predominates, histological [4] and biochemical evaluation [5] reveal that both processes are greatly accelerated in the affected bone. Although a general phenomenon, this is most apparent radiographically in the appropriately termed mixed lesions, seen quite commonly in breast cancer, in which both lytic and sclerotic components are clearly visible.

It is generally accepted that sclerosis of lytic metastases with no radiological evidence of new lesions constitutes tumour regression (a partial response or PR). Confounding factors include the appearance of sclerosis in an area which was previously normal on the radiograph. This may represent progression of a new metastasis, but equally can indicate a response, reflecting a radiographic example of the healing flare phenomenon within a lesion which was present at the start, but was not destructive enough to be radiologically visible [1–6]. Interpretation is further complicated by variations in film exposure and the effects of overlying bowel gas. The evaluation of response in osteosclerotic lesions is even more difficult with most patients with sclerotic metastases eventually classified as either 'no change' (NC) to therapy or as unassessable. Here, decisions about the efficacy of treatment have to be based on symptomatic response or (when present) change in extraskeletal disease.

Although the plain radiograph remains as the assessment tool for judging response in clinical trials, it is clearly an inadequate technique. This lack of precision for radiographic

assessment of response is exemplified by the observation that patients with X-ray evidence of sclerosis and those with no change in radiographic appearance for at least 3 months, have a similar outcome in terms of survival [7].

Radionuclide imaging

The radionuclide bone scan is well established as the most sensitive method for detecting pathological change in the skeleton. The bone-seeking radio-pharmaceutical is adsorbed on to the calcium of hydroxyapatite in bone, a reaction which is influenced by the osteoblastic activity and skeletal vascularity, with preferential uptake of tracer at sites of active bone formation. The bone scan, therefore, reflects the metabolic reaction of bone to the disease process, whether neoplastic, traumatic or inflammatory. When bone metastases develop, there is usually sufficient increase in local blood flow and reactive new bone formation to produce a focal increase in tracer uptake, often before bone destruction can be seen radiographically.

The use of bone scanning for assessment of response to therapy has always been contentious and certainly when lytic metastases predominate is often unreliable. A reduction in the intensity and number of lesions (hot spots) on the bone scan was previously considered to represent response and progressive disease assumed if an increase in intensity or number of hot spots was seen. However, this interpretation is too simplistic.

Following successful therapy for metastatic disease, the healing processes of new bone formation cause an initial increase in tracer uptake, akin to callus formation and scans performed during this phase (< 6 months) are likely to show increased intensity and number of hot spots. After treatment for 6 months, the bone scan appearances may improve as the increased production of immature new bone, the cause of the hot spots, eventually ceases and isotope uptake gradually falls. This 'deterioration' followed by subsequent 'improvement' in the bone scan appearances following successful therapy has been termed the flare response and is now a well-recognised phenomenon in breast [6, 8] and prostate cancer [9]. Additionally, a reduction in isotope uptake can occasionally be seen in rapidly progressive disease when the overwhelming bone destruction allows little chance for new bone formation, sometimes culminating in a photon deficient (cold spot) lesion on the bone scan. Bone scanning in advanced disease should certainly be interpreted with great caution when performed within 6 months of a change in therapy and is of most use for re-staging at the time of relapse, to identify sites for radiological assessment and bring to the attention of the clinician those sites at risk of pathological fracture where prophylactic surgery may be indicated.

Computed tomography (CT)

CT offers three-dimensional information and high quality images. The density discrimination is far superior to that found with conventional radiographic examination, providing excellent bone to soft tissue resolution. Bone destruction can be identified early and extra-osseous, soft tissue and intra-osseous medullary spread assessed. When the marrow cavity is replaced by tumour, higher attenuation of the X-ray beam is observed in comparison with the affected marow [10].

CT is most appropriate for diagnosing metastases, particularly in the spine, but it is also occasionally valuable as a parameter of response to metastatic disease. Metastatic lesions are selected which are considered to be representative of the metastatic process (target lesions) and suitable for serial examination. Because of the need for accurate re-positioning of the patient, only target lesions in the spine and proximal ends of humerus or femur should be used. 3–4 mm thick transverse sections with a standard window width and level appropriate for bone are optimum. If required, a region of interest (ROI) can be defined and the spectrum of Houndsfield values within it calculated by the computer. Changes in the spectrum with time can be determined, with a shift to the right (more positive) indicating replacement of lytic bone, which has a low Houndsfield value, by new sclerotic bone, which has a relatively high Houndsfield number. In one small prospective study, CT correlated with clinical assessment in 13 (65%) patients whereas correlation between plain radiographs of the target lesion and clinical assessment of response was obtained in only 10 patients (50%) [11].

CT is particularly useful for assessing areas such as the sacrum which are poorly visualised on plain radiographs and may enable assessment of sclerotic bone disease. Within sclerotic metastases, areas of osteolysis are usually present and may be identified by CT. Sclerosis of this lytic component would suggest response to treatment while new areas of lysis appearing within a sclerotic region probably represents progression [12].

Magnetic resonance imaging (MRI)

MRI is rapidly becoming more widely available and is an excellent imaging modality for the detection of bone metastases [13]. Unlike CT where it is impractical to image more than a limited part of the skeleton, sagittal MRI views allow large sections of the skeleton to be assessed. Cortical bone does not produce a signal and appears black on MRI but due to its fat content, normal bone marrow shows as a high intensity signal on T1 weighted sequences. Bone metastases produce a reduced signal, reflecting replacement of marrow fat. This allows early diagnosis of skeletal metastases [14] and is of value in distinguishing metastatic and osteoporotic vertebral collapse in post-menopausal women with breast cancer [15].

The use of MRI for monitoring response to treatment is at present anecdotal. However, because of limited machine availability, MRI is unlikely to be suitable for routine use but, as in primary bone tumours, may have a role for detailed evaluation of a specific lesion, for example prior to surgery.

BIOCHEMICAL MONITORING

Metastatic bone destruction results from the invasion of malignant cells from the bone marrow cavity. These cells secrete a variety of paracrine factors which stimulate bone cell function. Of particular importance is the stimulation of osteoclast function resulting in osteolysis and disruption of the normal coupling between osteoblast and osteoclast function [16]. This in turn leads to changes in a variety of biochemical parameters. When treatment is prescribed for a patient with bone metastases, the effects of that treatment on the tumour cell population will influence bone cell activity. These changes can be appreciated within the first few weeks of starting effective therapy and, therefore, biochemical markers which reflect the rates of bone formation and resorption, respectively, could provide an early assessment of response to treatment (Table 1).

Table 1. *Biochemical markers of bone resorption and formation*

Resorption	Formation
Urine	Serum
Calcium	Alkaline phosphatase
Hydroxyproline	Osteocalcin
Pyridinoline	PICP
Deoxypyridinoline	PIIINP
Ntx	
Crosslaps	
Free Dpd	
Free Pyr	
Galactosyl hydroxylysine	
Serum	
Calcium	
ICTP	
Galactosyl hydroxylysine	

Dpd, deoxypyridiniline; Pyr, pyridinoline; Ntx, N-telopeptide of type 1 collagen; ICTP, Type 1 collagen C telopeptide; PIIINP, Procollagen type III propeptide; PICP, propeptide of type 1 procollagen.

Markers of bone formation

Type 1 collagen is the major protein of bone and accounts for approximately 90% of the organic matrix. It is synthesised by osteoblasts to enable bone matrix deposition as a large precursor protein, Type 1 procollagen. Assay of the carboxy terminal propeptide of Type 1 procollagen (PICP) is now possible by radioimmunoassay and is believed to be a marker of early bone formation, appearing principally during the phase of osteoblast proliferation [17, 18].

Osteoblasts are naturally rich in alkaline phosphatase and release of the enzyme into the circulation, predominantly in the middle stages of bone formation during the matrix maturation phase, gives some indication of osteoblast activity. Measurement of total alkaline phosphatase is routine, but to exclude the contribution from the liver and other organs, bone isoenzyme estimation (ALP-BI) is required. Raised levels reflect increased new bone formation and, in oncology, the highest values are found with osteoblastic metastases or in response to healing [19].

Osteocalcin (BGP) is a marker of the late phases of bone formation appearing during the mineralisation phase [18]. It is also synthesised in osteoblasts, and contains three residues of the vitamin K dependent amino acid γ-carboxy glutamic acid. Osteocalcin binds strongly to hydroxyapatite, but a small fraction of the newly synthesised protein appears in the circulation from which it is rapidly cleared by the kidney. Measurement of serum levels is possible by a variety of radioimmunoassays.

Markers of bone resorption

Resorption of bone releases calcium, hydroxyproline and collagen fragments, all of which may be useful markers of response to treatment. Serum calcium measurements are routinely performed, but changes within the normal range give little guide to disease activity. Hypercalcaemia is usually indicative of progressive disease and certainly this is the case if the hypercalcaemia has developed more than a few weeks after the start of a new systemic treatment. Rarely, hypercalcaemia can be a manifestation of the tumour flare, occurring within a week or so of starting tamoxifen and this may herald a response to treatment [20]. Hypocalcaemia is seen when osteoblastic metastases predominate. This is more typically associated with prostate cancer, but does sometimes occur in advanced breast cancer.

Urinary calcium excretion is a more sensitive indicator of alterations in calcium homeostasis. The molar ratio of calcium to creatinine in an early morning urine sample collected after an overnight fast is a convenient reproducible method of quantifying calcium excretion. Initially reported as a marker of response more than 10 years ago [21], follow-up studies have confirmed this with a rapid fall in urinary calcium excretion typical of response. The problem with urinary calcium is that it is not a specific resorption inhibitor, but reflects the net effects of bone formation and resorption, is influenced by diet, the circulating levels of both parathyroid hormone (PTH) and parathyroid related protein (PTHrP) and the concommitant administration of drugs such as bisphosphonates which influence bone resorption independently of any tumour related effects [22].

Urinary hydroxyproline excretion is a conventional parameter for measuring bone resorption in benign bone disease but has been generally unreliable in the monitoring of metastatic bone disease due to the contribution from both diet and the soft tissue destruction by metastases [22, 23].

It is hoped that the recently introduced measurements of the intermolecular cross-linking compounds of collagen could overcome the poor specificity of the old markers. Pyridinoline (Pyr) and deoxypyridinoline (Dpd), also called hydroxy-lysylpyridinoline and lysyl-pyridinoline, respectively, are two cross-linking amino acids which hold together the extracellular telopeptide region at the ends of adjacent collagen chains. Pyr is found in bone, cartilage and to a lesser extent other connective tissues, while Dpd is almost exclusive to bone. They can be measured accurately in urine by reverse phase high performance liquid chromatography (HPLC) and have been reported as specific measures of the rate of bone resorption [24].

Although both Pyr and Dpd have provided useful information in benign and malignant skeletal conditions, the HPLC assays are too complex and time-consuming to be suitable for routine laboratory practice. Cross-links exist in both free (40%) and peptide-bound forms (60%) and recently, new enzyme-linked immunoassays (ELISA) have been developed to measure the protein-bound crosslinking molecule at either the N-terminal part (NTx) [25] or the C-terminal [26] part (Crosslaps) of type I collagen and the free portions of both pyridinoline (F-Pyr) and deoxypyridinoline (F-Dpd) [27], thereby providing a range of relatively simple assays for specific assessment of the rate of bone resorption. Our own studies in hypercalcaemia of malignancy have shown that the simpler ELISA assays correlate closely with the HPLC based Dpd and Pyr measurements and reflected the clear clinical differences observed between treatment with pamidronate or clodronate for hypercalcaemia of malignancy [28].

There is also great interest in developing a reliable serum assay for cross-links. At present the only available serum assay is the C-telopeptide cross-links (ICTP) developed by Ristelli and associates [29]. This assay, unlike the Crosslaps assay for urine, recognises elements from the helical part of both the α1 and α2 chains. Its clinical value is uncertain as, in clinical situations where resorption rates are known to be profoundly affected by treatment, ICTP levels change very little [22, 30].

Tumour markers

Unlike the germ cell malignancies, there is not a highly specific tumour marker for either diagnosis or monitoring of disease. However, some breast tumours do produce tumour antigens which can be detected by radioimmunoassay. The most widely studied are carcino-embryonic antigen (CEA) which is elevated in 50–80% of patients with metastatic breast cancer and CA 15-3 which is elevated in 60–90% of cases. Levels of CA1 15-3 are elevated more commonly than CEA, particularly when there is metastatic disease and serial measurements appear to correlate closely with response in measurable disease [31]. Although it is clear that CA15-3 and CEA are not useful for screening for metastases, a detailed prospective evaluation of their routine use in advanced breast cancer does seem justified. There are also a number of other mucin markers which are potentially of value and it is probably realistic to expect that a panel of tumour markers will be identified which collectively can provide reliable information on tumour response.

SUBJECTIVE RESPONSE ASSESSMENT

The relief of symptoms is the principal aim of palliative therapy and rationally should be the most important marker of response to treatment. However, the use of pain as a marker of response in clinical trials has not found universal acceptance and there is still no internationally accepted pain questionnaire in oncology. Subjective response to treatment for bone disease requires information on pain intensity, analgesic consumption and mobility, all of which need to be recorded. A simple questionnaire which amalgamates these three components has been adopted by us for use in a number of recent trials of bisphosphonate therapy for metastatic bone disease [1]. Validation of this questionnaire in terms of close correlations with objective response to treatment [32], quality of life measurement and more complex pain assessment methods [33] has been demonstrated.

Quality of life assessment is now an important aspect of clinical trials methodology and, notwithstanding all the difficulties of analysis and interpretation, well validated tools such as the Rotterdam Symptom Checklist [34] and the EORTC QOL-C30 [35] questionnaire can provide useful information on subjective response to treatment in the routine clinical setting.

BIOCHEMICAL MARKERS FOR DIAGNOSIS OF BONE METASTASES

Bone formation markers

Osteocalcin levels are significantly increased, compared with those in normal subjects, in metastatic bone disease from breast and prostate cancers [36], but not in multiple myeloma [37]. Levels are significantly higher in patients with blastic rather than lytic metastases and show some correlation with tumour bulk in prostate cancer [38]. Similar trends are reported with ALP-BI with highest levels in patients with multiple blastic metastases [19]. Correlation between ALP-BI and osteocalcin is variable, reflecting their relationship to different phases of the bone formation process [18]. Neither ALP-BI or osteocalcin are useful for early diagnosis of bone metastases.

PICP levels typically correlate well with ALP-BI, especially for patients with blastic metastases [19]. Increased levels are seen in over half of patients with prostatic metastases [39] and around 25% with breast cancer [19], but is usually normal in multiple myeloma [40].

Bone resorption markers

Urinary calcium excretion is not significantly increased in the majority of patients with metastatic bone disease [22], reflecting the effects of bone formation and renal handling of calcium. Hydroxyproline excretion, which indicates matrix destruction more specifically, is typically increased in breast cancer [23, 32] and myeloma but variable results have been found in prostate cancer [41].

It is clear that the collagen cross-links are much more reliable in confirming metastatic bone disease. In a review of 6 studies, elevated levels of Pyr and Dpd were reported in 58–88% and 60–80%, respectively [22]. In breast cancer, levels of Pyr and Dpd have also been slightly elevated in breast cancer patients without evidence of skeletal metastases, perhaps indicating sub-clinical involvement or, more likely, the effects of PTHrP and other bone mobilising tumour products on the skeleton [42].

MONITORING EFFECTS OF BISPHOSPHONATES IN METASTATIC BONE DISEASE

Bisphosphonates are an important new treatment modality in the management of metastatic bone disease. Through their potent inhibitory effects on bone resorption they have become the treatment of choice for hypercalcaemia of malignancy [43] and are able to reduce the number and rate of skeletal complications in multiple myeloma [44] and advanced breast cancer [45], delay the onset of progressive disease in bone following palliative chemotherapy for both breast cancer and myeloma [46, 47] and relieve metastatic bone pain caused by a variety of solid tumours with a consequent improvement in quality of life [48].

The effects of bisphosphonates on bone pain are now well recognised. To obtain optimal analgesic effects, the intravenous route is necessary, at least until more potent and well tolerated oral bisphosphonates have been developed. The majority of intravenous studies have been with pamidronate, but intravenous clodronate can also relieve pain [49]. Quite consistently, the phase II studies have reported relief of bone pain in around half the patients [43]. However, the reason(s) for a lack of symptomatic response in the other 50% of patients treated are unclear. Bone pain is a complex process and mechanical factors such as spinal instability or nerve entrapment may contribute. Nevertheless, much of the morbidity associated with bone metastases is secondary to focal tumour-induced osteolysis.

Changes in cross-link excretion have been evaluated in patients receiving bisphosphonate treatment for hypercalcaemia of malignancy. Before treatment, Dpd and Pyr excretion is four times the upper limit of normal and the levels of the peptide-bound crosslinks (Ntx and Crosslaps) increased up to 7 times normal [28]. After treatment, with full doses of either pamidronate [28, 50], clodronate [28] or ibandronate [51], levels of Pyr and Dpd fall by 30–60% while after pamidronate or clodronate, the reduction in Ntx and Crosslaps is 80–90% [28, 50].

Cross-link excretion has also been used to monitor response to bisphosphonates in normocalcaemic subjects with significant falls of 40–50% in Dpd and 25–35% in Pyr following either intravenous [52] or oral [53] pamidronate for metastatic breast cancer and oral residronate for multiple myeloma [37].

We have performed two studies in 86 normocalcaemic patients with heavily pretreated painful progressing bone

metastases (52 breast, 17 prostate, 17 others) to investigate a possible link between inhibition of bone resorption and symptomatic response. Patients received pamidronate 120 mg as a 2 h outpatient infusion. The first study ($n = 34$) was an open uncontrolled phase II evaluation of a single infusion, but for patients who reported benefit retreatment was given when the pain increased again [32]. In the second study [54], 52 patients were randomised to receive either a 2 h infusion of pamidronate 120 mg in 1000 ml 0.9% saline infusion or an identical infusion of saline alone. Four weeks later, or earlier in the event of worsening symptoms ($n = 13$), all patients received an infusion of pamidronate 120 mg. Thereafter patients experiencing subjective benefit received further infusions when their symptoms worsened again.

Patients had received at least one previous systemic anticancer treatment. No concomitant systemic anticancer treatment or radiotherapy during the course of the study was allowed. Analgesics were prescribed as necessary. To assess the effect of treatment on the rate of bone resorption, a second voided morning urine sample was collected every 2 weeks and stored at $-20^{\circ}C$ for measurement of collagen crosslinks by either HPLC or ELISA assays.

In the first study, total Pyr and Dpd were measured after acid hydrolysis, CF1 cellulose column partition chromotography and reverse-phase HPLC with fluorescence detection. In the second study, the easier and more sensitive ELISA assays to the N-terminal (Ntx) and C-terminal peptide-bound and free portions of the collagen crosslinks were measured. NTx was measured using the Osteomark™ kit (Ostex®, Inc, Seattle, Washington, U.S.A.), the C-telopeptide of type I collagen by the Crosslap™ assay (Osteometer A/S®, Rodovre, Denmark), and free deoxypyridinoline (free Dpd) by the Pyrilinks-D™ (Metra Biosystems®, Mountain View, California, U.S.A.) assay. In both studies, urinary calcium and hydroxyproline (Hyp) were measured by colourimetric assays. All the urinary markers results were expressed as a ratio to creatinine excretion in the urine.

Prior to commencing therapy and then weekly for 12 weeks, patients were asked to complete a pain intensity (P) and analgesic consumption (A) questionnaire and their WHO performance status (P) was recorded. These three parameters were combined to produce an overall pain score (PPA) as described previously [32]. The pain score was expressed as the percentage change from baseline. Clinical response to treatment was defined as $\geq 20\%$ decrease in PPA recorded on at least two consecutive measurements. Patients with a decrease in PPA of $< 20\%$ were considered non-clinical responders.

In the first study the mean reduction in PPA after a single infusion was 25% (standard error (S.E.) 3%, range 0–75%). 20 patients (59%) showed a confirmed $\geq 20\%$ improvement in PPA and were classified as responders [33]. In this study, the first evidence in oncology to suggest a link between metastatic bone pain and bone resorption was seen [55]. Subjective response correlated with biochemical response; patients showing a $\geq 50\%$ reduction in Dpd were more likely to respond than those showing a $< 50\%$ fall in this specific marker ($P < 0.05$). In addition, as patients returned requesting further treatment because of an increase in bone pain, the levels of Dpd were noted to be rising at this time.

In the second study, during the initial 4 week placebo-controlled phase, a significant reduction in PPA was seen after pamidronate ($P = 0.03$) but not with placebo. Within 4 weeks of the second infusion (all active pamidronate), a total of 22 patients (53%) had achieved a symptomatic response. Both Ntx and Crosslaps fell to a mean of 25% of baseline after pamidronate but were unaffected by placebo ($P < 0.01$).

The pretreatment NTx values in this study predicted clinical response to treatment. The baseline NTx of the clinical non-responders was significantly higher (199 ± 40 nmol BCE/mmol) than the baseline value of the clinical responders (102 ± 16 nmol BCE/mmol); ($P < 0.02$). Patients with an initial NTx value more than twice (> 2) the upper normal value rarely showed a response to therapy (2/16, 13% response rate), despite a 70% fall in NTx values, while clinical response was frequent (17/27, 63% response) in those patients with pretreatment levels of NTx less than twice (< 2) the upper normal value.

Additionally, patients in whom the rate of bone resorption, as measured by NTx, Crosslaps or Free Dpd did not return to normal after pamidronate had a very poor clinical response (0–20% depending on the marker), while in those patients in whom a biochemical response was achieved or with marker levels always in the normal range, the frequency of response was much higher (53–63%, again depending on the marker—$P < 0.01$; Table 2).

These results suggested that patients with bone metastases and a very high rate of bone resorption respond poorly to pamidronate, even at the fairly high-doses used in these studies and that patients likely to benefit from treatment could be selected on the basis of biochemical measures of bone resorption, clinical benefit being greatest for those with slowly progressive disease and only modest increases in the rate of bone resorption. A high rate of bone resorption appears to be one of the factors underlying resistance to bisphosphonates. It has been suggested that as bisphosphonates are taken up preferentially at active bone surfaces, the bisphosphonate will be distributed across more areas of increased bone resorption in patients with more severe disease and thus, the amount deposited in each individual lesion will be less. For patients with more aggressive disease, more potent bisphosphonates, a higher-dose or dose-intensity, or combined anticancer therapy and bisphosphonate treatment may be required.

BIOCHEMICAL MARKERS FOR RESPONSE ASSESSMENT

In the mid-1980s there were several studies which investigated the use of biochemical assessment of response to systemic therapy [23, 32, 56]. These generally showed that radiological response is correlated with a reduction in bone resorption. However, perhaps because at the time urinary

Table 2. Influence of biochemical response on probability of clinical response to intravenous pamidronate

		Clinical response (%)
Ntx response	Yes	12/19 (63)
	No	0/11 (0)
	Always normal	7/13 (54)
Crosslaps response	Yes	13/24 (54)
	No	0/7 (0)
	Always normal	6/12 (50)
Free Dpd response	Yes	8/15 (53)
	No	3/15 (20)
	Always normal	8/13 (62)

Yes, raised pretreatment level returned to the normal range. No, raised pretreatment level failed to return to the normal range.

calcium and hydroxyproline were the only available resorption markers, the reliability of biochemical monitoring was not established.

In one prospective study of 70 unselected patients with advanced breast cancer receiving systemic treatment [32], 15 out of 16 responders (94%) had a >10% reduction in calcium excretion compared with 10 out of 21 (48%) with progressive disease ($P < 0.01$). Markers of osteoblast activity were also evaluated in this study. After 1 month, 15 out of 16 responding patients (94%) showed a >10% rise in both ALP-BI and osteocalcin compared with 7 out of 22 (32%) with radiological evidence of progression ($P < 0.001$). The serum concentration of ALP-BI and osteocalcin subsequently fell steadily after 1–2 and 3–4 months, respectively. Variable changes in bone formation have been reported by others, depending on the timing of the sample collection and the confounding changes induced by the healing flare [56].

Recently, with the development of the cross-link assays, there has been renewed interest in biochemical monitoring [57]. A preliminary report of Dpd and Pyr measurements in breast cancer patients receiving endocrine treatment has shown that pretreatment levels of Dpd and Pyr were 2- to 3-fold higher in patients who progressed on treatment compared with stable or responding patients, whilst during treatment, crosslink excretion increased during progressive disease (PD), but remained stable or fell in the responders [58].

In prostate cancer, effective endocrine treatment leads to normal levels of Dpd, but in 13 out of 15 patients with PD, increased levels of Dpd were found [59]. In myeloma, one study has suggested that serial measurements of the serum resorption marker ICTP could discriminate between PD and disease response or stabilisation [40]. In this study, PICP was also measured. Multiple myeloma is a condition characterised by a depressed bone formation and, not surprisingly, levels of PICP were low to normal before treatment, reflecting this inhibition of osteoblast function, and remained so despite a reduction in tumour mass. In another study of patients with prostate cancer, PICP levels were elevated at study entry due to overactivity of osteoblasts [39]. Levels fell during treatment, but the significance of this change was difficult to interpret. Initially the fall may have been due to a reduction in both tumour burden and removal of tumour stimulation of osteoblast function. However, as patients started to progress again, levels continued to fall and at this stage in the illness this was attributed to enhanced catabolism and tumour-induced cachexia.

We have recently assessed biochemical response markers in 37 patients with radiologically confirmed bone metastases from breast cancer who took part in an EORTC multicentre, randomised, double-blind trial (10924) [60]. The bone metastases had been identified within the previous 6 weeks and patients were randomised to receive oral pamidronate 300 mg daily or identical placebo tablets in addition to standard anticancer treatment.

A fasting morning serum sample and a second voided urine sample were collected and radiographs of involved sites performed at baseline, 4 months, at 3 monthly intervals thereafter and at each time there was either a change in systemic therapy or a skeletal-related event. Radiological response to treatment was evaluated according to the UICC criteria [2] and all radiographs were reported by the same investigators without knowledge of the biochemical markers levels. Subjective response to treatment was evaluated according to the PPA pain score, as described above [32, 33]. The bone resorption markers measured were urinary calcium, urinary hydroxyproline and urinary N-telopeptide peptide-bound crosslinks (NTx) excretion. The tumour markers measured were CA 15.3 and CASA (cancer associated serum antigen).

Following the start of a new systemic treatment, there was a significant difference at 1 ($P < 0.05$) and 4 months ($P < 0.01$) in the mean NTx levels of those who progressed on treatment (PD) compared with patients showing no change (NC) or partial response (PR) patients. Ntx was also the only bone resorption marker able to discriminate reliably between patients progressing early on in treatment (time to progression (TTP < 7 months) from those with longer disease control (TTP > 7 months). In PD patients, Ntx levels steadily rose and Ntx was the best marker for identifying progressive disease; the diagnostic efficiency of a 50% increase in Ntx for identifying imminent progression was 78%. Hydroxyproline only showed a difference between these groups at 4 months, while uCa could not differentiate between them. For the other markers evaluated, there was a significant difference in hydroxyproline and CA 15.3 levels between PD and NC or PR patients at 4 months ($P \leq 0.05$), but not at 1 month and no significant difference at either time point with urinary calcium or CASA. The conclusion from this study was that Ntx is the most promising marker for monitoring bone response and that further prospective evaluation was indicated.

There is only one other report on the use of collagen cross-link measurements following systemic therapy associated with bisphosphonates [61]. In this trial, 51 patients (49 breast cancer, 2 myeloma) with bone metastases were treated with either pamidronate 90 mg i.v. monthly or placebo in addition to standard systemic therapy. During the 6 months of the trial, NTx was the marker that showed the most significant decrease ($P = 0.0006$), while there was a smaller difference with deoxypyridinoline ($P = 0.03$) and no detectable difference with pyridinoline.

CONCLUSION

Serial plain radiographs are inadequate for monitoring the response of bone metastases to treatment. There are now reliable, specific measures of bone resorption and formation which are relatively simple to measure. The use of modern biochemical markers of bone metabolism, tumour markers, subjective response and quality of life assessment needs continued refinement but in the future is likely to provide more clinically and biologically relevant information than has been available hitherto.

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