Multiple Myeloma • Research Paper

Sequential analysis of biochemical markers of bone resorption and bone densitometry in multiple myeloma

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A B S T R A C T

Background and Objectives. Bone lesions often occur in multiple myeloma (MM), but no tests have proven useful in identifying patients with increased risk. Bone marker assays and bone densitometry are non-invasive methods that can be used repeatedly at low cost. This study was performed to evaluate these methods in predicting bone events in MM patients.

Design and Methods. Thirty newly diagnosed MM patients were enrolled. Serum C-terminal telopeptide (ICTP) and urinary N-terminal telopeptide (NTx) of collagen I were measured for assessment of bone resorption, and serum C-terminal (PICP) and N-terminal (PINP) propeptides of procollagen I, bone-specific alkaline phosphatase, and osteocalcin were measured to estimate bone formation. Dual energy X-ray absorptiometry (DEXA) was used to assess bone mineral density (BMD) of the lumbar spine, hip, and whole body. Serum and urine samples were collected every 6 weeks, DEXA-scans performed every 3 months, and skeletal radiographs were done every 6 months as well as when indicated.

Results. Serum ICTP and urinary NTx were predictive of progressive bone events. Markers of bone formation, bone mineral density assessments, and M component measurements were less informative. In Cox analysis, ICTP showed the highest predictive value, but should be replaced with NTx in patients with nephropathy. Pretreatment low lumbar BMD was predictive of early vertebral fractures.

Interpretations and Conclusions. Sequential DEXA-scans showed heterogeneous local BMD changes, and our data do not support routine use of sequential DEXA-scans. However, lumbar DEXA-scans at diagnosis can identify patients with increased risk of early vertebral collapses. Sequential analyses of serum ICTP and urinary NTx are useful for monitoring bone damage.

Key words: multiple myeloma, osteolytic bone disease, biochemical markers of bone metabolism, carboxy-terminal telopeptide of collagen type I (ICTP).

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The major clinical features of multiple myeloma (MM) are painful osteolytic destructions and pathological fractures. In collaboration with other cells in the bone marrow the myeloma cells induce increased osteoclast activation and osteoclastic bone resorption, whereas the effects on the osteoblasts are more complex.[1-4] Bone disease in MM is usually assessed by radiographs of the skeleton. Radiographs are useful in diagnosing of osteolytic lesions, but do not give information on ongoing degradative activity.

Biochemical markers of bone metabolism include assays for estimating bone resorption and assays for estimating bone formation. A histomorphometric study has shown that these markers reflect ongoing bone resorption and bone formation in patients with MM.[5] The markers of bone resorption have also been explored in a few clinical studies of patients with MM. Most of these studies have been cross-sectional studies showing that these markers are elevated in MM,[6] or longitudinal studies showing that they provide prognostic information on survival.[7-9] Follow-up measurements or registration of the bone disease were not included in these studies. Studies with sequential measurements of markers of bone resorption have either included few patients,[10,11] been short-term studies with no follow-up registration of bone morbidity,[12,13] or have addressed the biochemical response to different treatment strategies.[14-17] Recently, we have shown that elevated pre-treatment levels of the C-terminal telopeptide of collagen type I (ICTP) in serum and of the N-terminal telopeptide of collagen type I (NTx) in urine are predictive of early progression of bone disease in patients treated with standard chemotherapy.[18] No study, howev-

er, has shown whether these markers provide relevant information for daily clinical practice concerning the development of bone disease over time in patients with MM.

Bone mineral density (BMD) can be assessed by dual energy X-ray absorptiometry (DEXA). Our preliminary experience with DEXA has shown that BMD of the lumbar spine is often diminished in MM patients at diagnosis, whereas BMD of the hip is rarely affected.[19] Furthermore, a low BMD in the lumbar spine correlated to the severity of the radiological findings at diagnosis.[19] Sequential BMD measurements in MM have only been reported in a few studies,[20-24] and these studies indicate that DEXA can provide important information concerning bone disease in MM.

We hypothesized that clinical and radiological bone lesions are preceded by a period of increased bone resorption and loss of bone mass. In the present study we explored the clinical usefulness of biochemical markers of bone metabolism and DEXA in predicting bone events and disease progression in patients with MM.

Design and Methods

Patients

Thirty consecutive patients with newly diagnosed symptomatic MM were enrolled in this study from 1992 to 1997. There were 15 women (median 71 years, range 44-83) and 15 men (median 70 years, range 51-80). Most patients had advanced disease according to the Durie & Salmon staging system (stage II: 8 patients; stage III: 22 patients).[25] Twenty-two patients had the IgG isotype, four patients the IgA isotype, three patients had light chain disease, and one patient non-secretory disease. Ten patients presented with hypercalcemia (serum calcium corrected for serum albumin $\geq$ 2.60 mmol/L), and four patients had serum creatinine levels above 120 mmol/L.

Two patients had normal radiographic findings of the skeleton. All other patients had lytic bone lesions, pathological fractures, or both.

None of the patients had received treatment with corticosteroids or bisphosphonates before entry into the study. Other exclusion criteria were previous or concomitant secondary malignant disease, liver disease, and rheumatic disease. All patients were treated with cyclic melphalan-prednisone (MP) given every 6 weeks until maximal response was obtained (at least 8 cycles) or until treatment failure. In the case of progressive disease after initial response, treatment with MP was re-instituted. Second-line chemotherapy included intravenous anthracycline and vincristine (VAD or NOP). None of the patients received high-dose chemotherapy, and none was treated with prophylactic bisphosphonates. Bisphosphonates, glucocorticoids and hyperhydration were used for treatment of hypercalcemia.

The study was approved by the local ethics committee, and performed in accordance with Helsinki Declaration II.

Assays and biochemistry

Serum and urine samples were taken at diagnosis after rehydration but before the start of treatment, and thereafter every 6 weeks before each treatment course. Samples were taken between 8 and 9 a.m. and serum was stored at -80°C. Urine samples were taken as a second-void spot urine after an overnight fast and stored at -20°C.

The C-terminal telopeptide of collagen type I (ICTP) in serum was analyzed by a radioimmunoassay (RIA) from Orion Diagnostica (Oulunsalo, Finland). The assay employs rabbit polyclonal antibodies detecting a cross-linked fragment liberated by metalloproteinase activity from the C-terminal telopeptide region of type I collagen.[26-28] A normal range for ICTP of 1.0 to 4.9 μg/L was established based on analysis of serum samples from 135 healthy individuals (aged 40-75 years). Intra- and interassay imprecisions, expressed as a coefficient of variance (CV), were below 6% and 8%, respectively.

The N-terminal telopeptide of collagen type I (NTx) in urine was measured by an ELISA inhibition assay[29] from Ostex (Seattle, WA, USA). The assay employs a mouse monoclonal antibody (MoAb) 1H11 that recognizes an epitope embedded in the N-telopeptide pyridinoline region of human type I collagen. Intra- and interassay CVs were 5% and 12%, respectively. NTx results were expressed as nmol bone collagen equivalent per mmol creatinine. The normal reference range was 10 to 130 nmol/mmol.

The N-terminal propeptide of procollagen type I (PINP) in serum was measured by a RIA from Farmos Diagnostica (Oulunsalo, Finland). The assay employs polyclonal rabbit antibodies detecting the intact amino-terminal propeptide of procollagen type I.[30] The intra- and interassay CVs were 4% and 5%, respectively. The normal reference range was 20 to 98 μg/L.

The C-terminal propeptide of procollagen type I (PICP) in serum was measured by a RIA from Farmos Diagnostica (Oulunsalo, Finland). The assay detects the carboxy-terminal propeptide of procollagen type I, a trimeric globular protein with a molecular weight of 100,000 Da.[31] The intra- and interassay CVs were 3% and 5%, respectively. The normal reference range was 50 to 210 μg/L.

Osteocalcin in serum was measured by a RIA modified from Price & Nishimoto[32] using rabbit antisera

against bovine osteocalcin. Intact purified bovine osteocalcin was bought from the Procter & Gamble Company (Cincinnati, OH, USA). Intra- and interassay CVs were 5% and 10%, respectively. The normal reference range was 4.2 to 80.6 μg/L.

Total alkaline phosphatase activity (ALP) in serum was measured spectrophotometrically using *p*-nitrophenylphosphate as a substrate. Bone isoenzyme alkaline phosphatase activity (boneALP) was determined by lectin-precipitation.[33] Intra- and interassay CVs for ALP were 3% and 5%, and for boneALP 5% and 7%, respectively. The normal reference range for boneALP was 4 to 113 U/L.

Serum and urinary M component, serum calcium, albumin, creatinine, liver transaminases, β_2-microglobulin (β_2m), blood hemoglobin, leukocyte and platelet counts were measured using routine methods.

Bone densitometry

Bone mineral density (BMD, g/cm^2) of the whole body, lumbar spine (L1-L4, antero-posterior view), and hip was measured by dual energy X-ray absorptiometry (DEXA) using a Hologic QDR-1000 scanner. The right hip was routinely examined except in three patients with either an osteosynthesis or hip prosthesis, in whom the left hip was examined. Collapsed lumbar vertebrae identified by the radiographs were excluded from analysis. A mean BMD value of the non-collapsed vertebrae (L1-L4) was calculated in these patients.

Sequential DEXA scans of whole body, lumbar spine, and hip were performed every 3 months for 24-30 months of follow-up or until death. BMD changes over time were expressed in percentages of baseline BMD.

In our laboratory the *in vivo* precision of the whole body measurements is 1%. The precision of antero-posterior BMD measurements of individual lumbar vertebrae is 0.6%, whereas the precision for the femoral neck is 1.0%.

Data from 289 healthy Danish volunteers (181 females and 108 males) aged 21-79 years were used for calculation of Z scores, which for each patient represent the number of standard deviations above or below the statistical mean for age- and sex-matched individuals.

Bone radiology and follow-up assessment

The severity of the bone disease at diagnosis was assessed by radiographs of the whole skeleton and scored semi-quantitatively.[19] The skeleton was divided into seven separate regions: cranium, cervical spine, thoracic spine, lumbar spine, pelvis, long bones, and *other bones*. *Other bones* comprised the ribs, sternum, clavicles, scapulae, and mandible. Each region was scored for lytic lesions according to the following staging system: no lesion was assigned score 0; one small (<10mm) lesion score 1; one large (>10mm) lesion score 2; multiple small lesions score 3; and multiple mixed small/large or large lesions score 4. The scores were added to give an overall *osteolysis score*. Similarly, a *fracture score* was calculated by adding scores for vertebral collapses and non-vertebral pathological fractures. Vertebral fractures were scored according to the degree of deformity (crushed, wedged or biconcave): less than 20% collapse was considered not pathological and assigned score 0; 20-40% collapse was given score 1; 40-60% collapse score 2; >60% collapse score 3. A non-vertebral fracture was assigned score 1.

The whole skeleton was re-examined every 6 months and when indicated by symptoms. A *bone event* was defined as follows: 1) development of new osteolytic lesions or more than 25% growth of existing lesions; 2) vertebral or non-vertebral pathological fractures. Progression of osteolyses normally defines progression of MM.[34] However, when observed alone without other signs of disease progression, new vertebral collapses or progression of vertebral collapses of the thoracic or lumbar spine are not diagnostic of disease progression in a patient with MM.[34] Therefore, we registered separately vertebral collapses of the thoracic or lumbar spine as a *vertebral fracture*, and other fractures or osteolytic lesions as an *osteolytic event*.

Bone disease was considered in regression when partial or complete recalcification of osteolytic lesions was observed.

Evaluation of the radiographs was performed blindly by one of the authors (JEK).

Efficacy of treatment and response criteria

The EBMT criteria for evaluating disease response and progression were applied.[34] Complete remission was not observed in any of the patients.

Statistical analysis

Data are presented as mean ± SEM or median (range) depending on whether their distribution was normal or non-normal. One-way and repeated measures analysis of variance (ANOVA), with the Kruskal-Wallis or the Mann-Whitney U-test as non-parametric alternatives, were used for analysis of differences between groups. The prognostic significance of laboratory data and clinical variables in predicting bone events was tested by the Cox proportional hazard model including time-dependent variables. Laboratory values were entered as continuous variables (cont) and when appropriate also as categorical variables (cat) with the upper (or lower) reference limit as the cut-point. The predictive value of the biochemical markers was tested in four settings: (i) pre-treatment

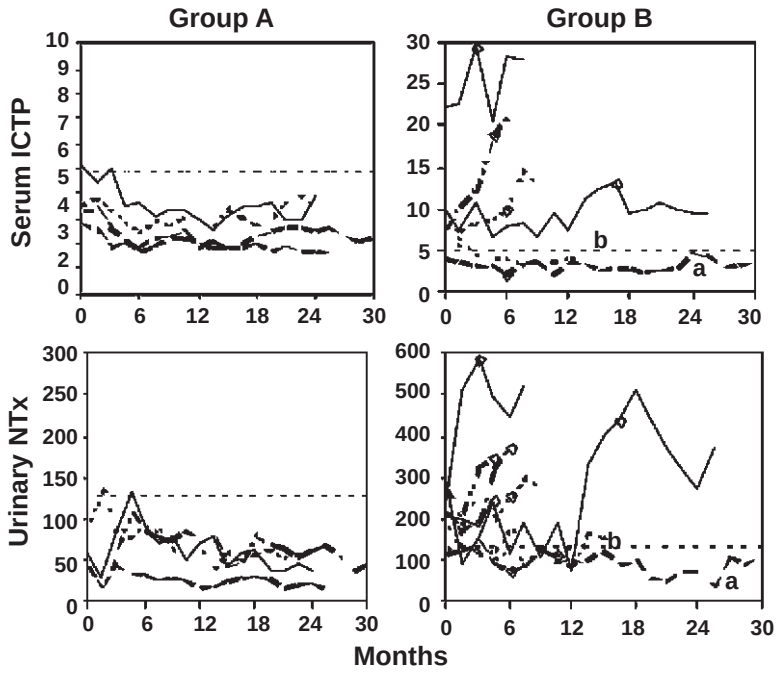

Figure 1. Serum ICTP and urinary NTx over time in multiple myeloma patients as a function of treatment response. Left: four patients with responsive disease or no change, and with no indications of disease progression within a follow-up period of 24 to 30 months (group A); right: nine patients with primary progressive disease. Time points of registered progression of osteolysis are marked by ◆. Data are not shown for ICTP in three patients with impaired renal function. The dotted lines indicate the upper reference limits for ICTP and NTx. One patient (case a) had osteolytic events but normal ICTP and NTx levels, whereas another patient (case b) with progressive disease and normal ICTP levels did not have osteolytic events before his death.

(base-line) level (cont/cat), (ii) the presence of an elevated level at a random time-point (cat), (iii) the presence of elevated levels in two consecutive measurements (cat), and (iv) as time-dependent variables (cont). The predictive value of BMD data were tested as (i) baseline Z scores, and (ii) time-dependent variables as per cent changes.

The most significant manifestation of each variable was included in the model. Significant prognostic factors were entered by forward selection. A p value less than 0.05 was considered statistically significant.

Because some variables (ICTP and osteocalcin) are dependent on renal function, we performed Cox analysis in two steps. First, we analyzed data for all patients including creatinine clearance values as an independent factor. Secondly, we analyzed data for patients with an estimated creatinine clearance greater than 50 mL/min, which involved excluding 4 patients from the analysis. Statistical analysis was performed using the SPSS 10.0/PC computer program (SPSS Inc. Chicago, IL, USA).

Results

Disease response to chemotherapy

Twenty-eight patients could be evaluated for response to treatment and their follow-up measurements analyzed. One patient was lost to follow-up and another patient died unexpectedly at home soon after the first admission. Thirteen patients fulfilled the criteria for partial response or minor response. Six patients had no change, and nine patients had primary progressive disease. Eleven patients with responsive disease and four patients with no change later had progressive disease.

The patients were followed for up to 30 months with a median observation time of 24 months. Bone events were registered in 24 patients. Osteolytic events, defining progression of multiple myeloma, were observed in 18 patients, and vertebral fractures were observed in 15 patients. Nine patients had both progression of osteolytic lesions and vertebral collapses.

Two patients with partial responses and one patient with a minor response had some radiologically assessed degree of healing of the lytic lesions.

Markers of bone resorption

Four patients had impaired renal function with estimated creatinine clearances < 50 mL/min. These patients had continuously elevated ICTP levels. The data on ICTP in these patients are not shown in Figures 1-3.

Serum ICTP and urinary NTx remained normal in patients with partial response, minor response or no change, and no later disease progression (Figure 1, group A).

Patients with primary progressive disease had elevated ICTP and/or NTx before development of osteolytic events in all but one case (Figure 1, group B). This patient had primary progressive disease with slow but steady progression of osteolytic lesions, increasing M component, and anemia, but had normal ICTP and NTx levels (case a in Figure 1). Another patient had disease

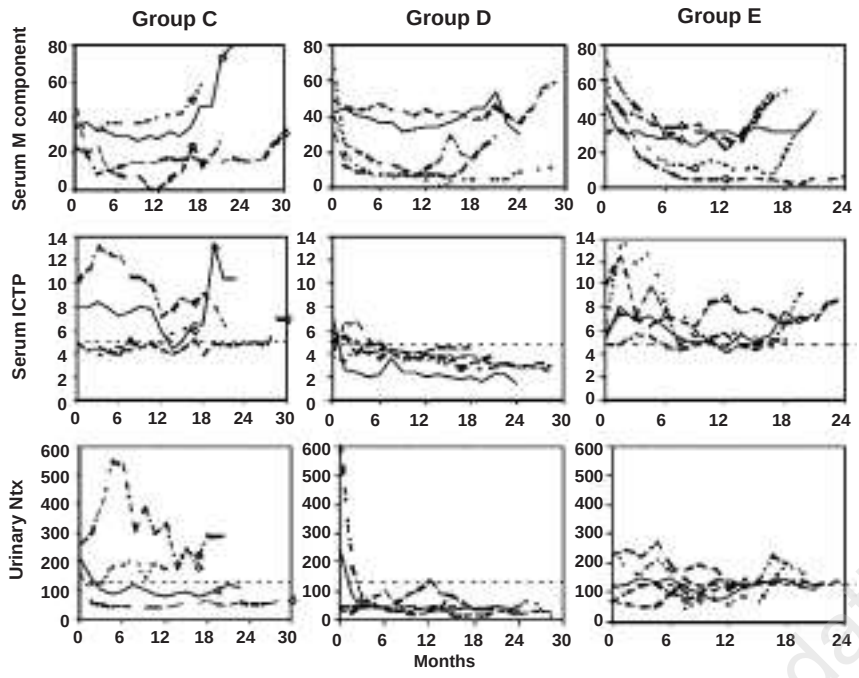

Figure 2. Serum M component, serum ICTP, and urinary NTx levels over time in multiple myeloma patients with progressive disease after initial response to treatment or no change. Left: four patients with progression defined by simultaneous increasing M component levels and osteolytic events (group C); middle: Five patients with progression defined by increasing M component levels but without osteolytic events (group D); right: five patients with progression defined by osteolytic events but without increments of M component levels until later in the disease course (group E). Time points of observed osteolytic events are marked by ◆. The dotted lines indicate the upper reference limits for ICTP and NTx.

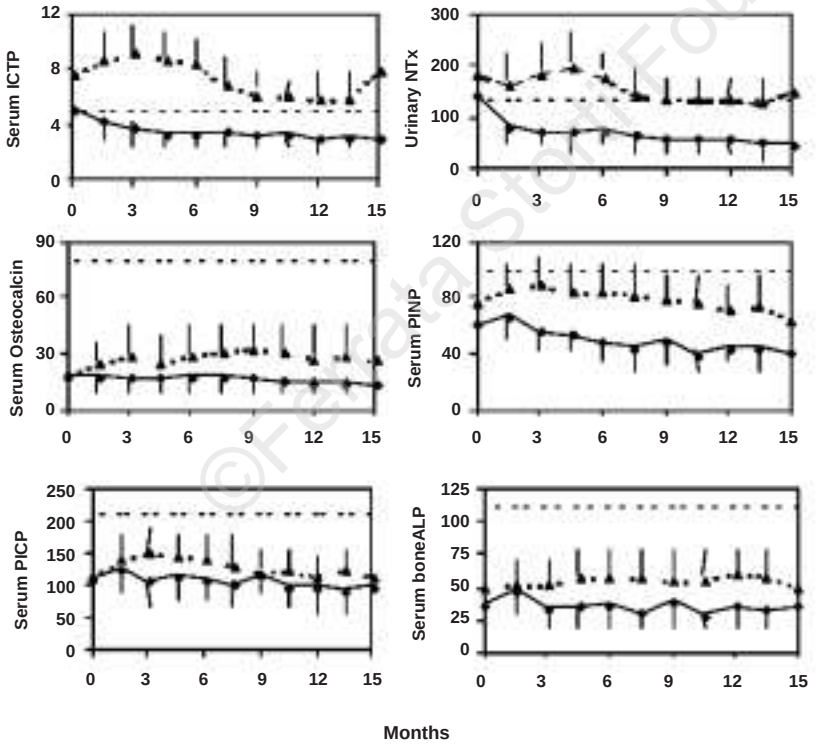

Figure 3. The levels of markers of bone resorption (ICTP and NTx), and of bone formation (osteocalcin, PINP, PICP, and boneALP), in 28 multiple myeloma patients over time. The continuous line (----) shows levels in 10 patients with no osteolytic events within an observation period of 24 months, and the interrupted line (- - -) 18 patients with osteolytic events. Values are shown as mean ± SEM. The dotted lines indicate the upper normal reference levels for the parameters.

progression with increasing M component and anemia, but no osteolytic events, until his death after 13 months. This patient had normal ICTP and only minor elevation of NTx (case b).

Most patients with initial response to treatment or no change, later had disease progression (Figure 2). Four patients had progression defined by simultaneous increasing levels of the M component and osteolytic events, and these patients had elevated and/or increasing levels of ICTP that almost paralleled the changes in the M component levels (Figure 2, group C). Five patients had progression defined by increasing M-component levels but did not develop osteolytic events, and these patients had normal levels of ICTP and NTx (Fig-

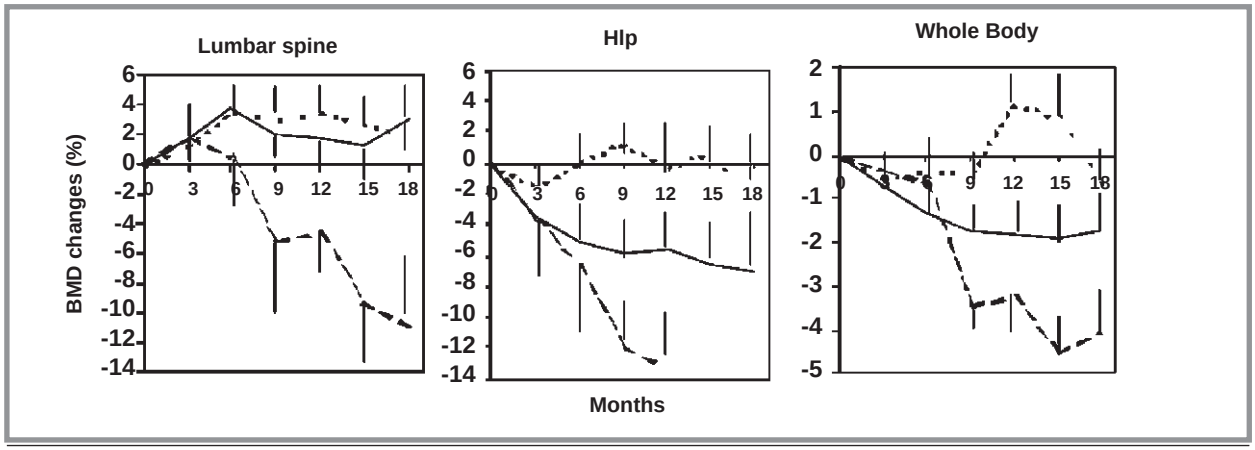

Figure 4. Changes in lumbar spine, hip, and whole body bone mineral density (BMD) changes over time in 28 multiple myeloma patients shown according to their patterns of response to pulse melphalan-prednisone treatment: 13 patients had minor or partial response (—), 6 patients had no change (- - -), and 9 patients had primary progressive disease (— — —). Patients with progressive disease had declining BMD as compared with the other groups ($p<0.01$). Patients with responsive disease had declining hip and whole body BMD as compared with patients with no change ($p<0.05$).

ure 2, group D). Five patients had disease progression defined by osteolytic events whereas the M component levels were stable until several months later (Figure 2, group E). These patients had elevated levels of ICTP, but not consistently elevated levels of NTx.

Patients with osteolytic events had higher levels of ICTP and NTx than did patients without osteolytic events (Figure 3). Patients with no osteolytic events responded to 6 months of MP treatment with a significant 33 % decrease in serum ICTP ($p<0.05$) and a 46 % decrease in urinary NTx ($p<0.05$).

Markers of bone formation

The serum levels of PINP, PICP, ostecalcin, and boneALP are shown in Figure 3. PINP showed more differentiated serum levels and changes over time than did PICP, osteocalcin, and boneALP. Few patients had elevated levels of PINP, and elevated levels of PICP, osteocalcin, and boneALP were seldom observed. Patients with osteolytic events had higher levels of osteocalcin, PINP, and boneALP at 3 months and thereafter than did patients with no events ($p<0.05$).

Bone densitometry

The changes in bone mineral density (BMD) as a function of response patterns are shown in Figure 4.

Patients with primary progressive disease had significantly decreasing lumbar spine BMD ($p<0.01$), hip BMD ($p<0.01$), and whole body BMD ($p<0.01$) as compared with patients with treatment response or no change. Patients with treatment response or no change did not have BMD changes over time in the lumbar spine, but patients who responded to treatment had significantly declining hip and whole body BMD as compared with patients with no change ($p<0.05$). Declining BMD in patients fulfilling criteria for partial or minor response were also seen for different specified areas within the scanned hip region (femoral neck, trochanter, inter-trochanter, and Ward´s triangle). However, as illustrated by the high SEM values in Figure 4, there were profound individual differences within the different response groups. Furthermore, several patients had a mixed BMD response to treatment, most often seen as increasing lumbar spine BMD during the same months as hip BMD was declining. In three patients the opposite pattern was noticed.

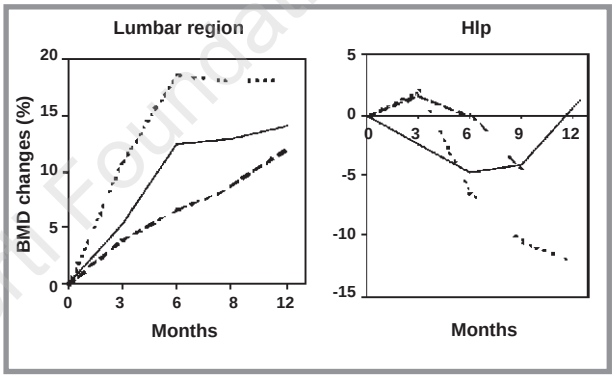

Figure 5. Left: lumbar spine BMD changes during the first year of treatment with pulse melphalan-prednisone in three multiple myeloma patients who responded to treatment as shown by regression of lytic lesions at radiographic examination after 6 and 12 months. DEXA-scans were performed every 3 months. Right: hip BMD changes in the same three patients.

Three patients had X-ray evidence of partial healing of osteolytic lesions after 6 and 12 months. Lumbar spine BMD increased markedly within a few months in these patients, as illustrated in Figure 5. As shown, hip BMD changes did not reflect the positive bone balance in these 3 patients.

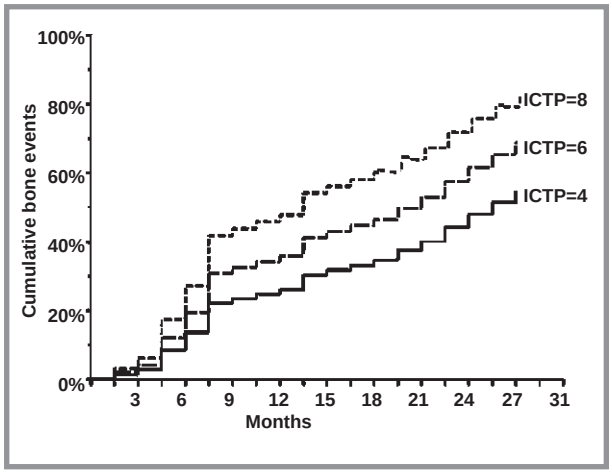

Figure 6. Cox model presentation of the predictive value of serum ICTP in multiple myeloma patients with creatinine clearances above 50 mL/min. The curves estimate the cumulative probability of a bone event in patients with different serum levels of ICTP (p=0.001).

Whole body BMD decreased significantly over time in patients who experienced osteolytic events in comparison with patients without osteolytic events. The change in whole body BMD at 12 months was –2.5± 0.6% in patients that developed osteolytic events vs. 0.16±0.7% in patients with no osteolytic events (p<0.01).

Prediction of bone events

In univariate analysis, a number of factors were significantly predictive for the development of bone events (osteolytic events and vertebral fractures) (Table 1). Baseline Durie and Salmon stage III disease, high serum β2 microglobulin, and severe skeletal disease were correlated with a higher risk of new bone events. Furthermore, elevated ICTP, NTx, and osteocalcin levels as time-dependent variables were predictive for development of new bone events. No significant predictive information was obtained from the measurements of serum M component, PINP, PICP, boneALP, or from the lumbar, hip, and whole body BMD changes over time.

In patients with normal renal function, ICTP showed the strongest significance and was the only factor to enter the multivariate Cox model. Inclusion of any other factor did not improve the model. An increment of serum ICTP by one unit (5 to 6 μg/L) observed in a patient over time, results in an approximately 18% increase in the relative risk of future bone events. The model is illustrated in Figure 6.

When patients with creatinine clearance < 50 mL/min were included, the predictive value of serum ICTP was diminished, and urinary NTx levels in combination with Durie & Salmon disease stage formed the most descriptive model (Table 1).

Table 1. Parameters with significant predictive value for bone events in 28 newly diagnosed multiple myeloma patients treated with standard chemotherapy.

	β	*hazard ratio*	*p*
Univariate analysis*			
Baseline data			
D & S stage III, categorical		5.83	0.005
Serum β2-microglobulin, mg/L	1.08	0.011	
Osteolysis score		1.14	0.007
Time dependent variables			
Serum ICTP, ug/L		1.16	0.02
Urinary Ntx, nmol/mmol creat	1.005	0.03	
Serum ICTP, ug/L#		1.18	0.001
Serum osteocalcin, μg/L#		1.026	0.033
Multivariate analysis°			
Urinary Ntx, nmol/mmol creat	0.004	1.004	0.036
D & S stage III, categorical	0.955	3.2	0.014
Multivariate analysis#			
Serum ICTP, μg/L	0.167	1.181	0.001

**Log-rank test; °Cox proportional hazard model; #analysis excluding four patients with creatinine clearance less than 50 mL/min are excluded; D& S, Durie& Salmon. The hazard ratio for a variable is the relative risk corresponding to the increment by 1 unit for the variable. The unit for stage is the step from D& S stage II to stage III of the disease.*

Table 2. Parameters with significant predictive value for vertebral fractures within the first year from diagnosis in 28 multiple myeloma patients treated with standard chemotherapy.

	β	*hazard ratio*	*p*
Univariate analysis*			
Baseline data			
Serum ICTP, μg/L		1.22	0.009
Urinary Ntx, nmol/mmol creat		1.008	0.018
Serum β2-microglobulin, mg/L		1.14	0.001
Vertebral fracture score		1.37	0.003
Lumbar spine BMD, Z-score		0.63	0.016
Multivariate analysis**			
Vertebral fracture score	0.367	1.33	0.001
Serum β2-microglobulin, mg/L	0.16	1.172	0.001

**Log-rank test; °Cox proportional hazard model.*

In the search for possible different predictive factors for vertebral fractures and progressive osteolysis, we subsequently analyzed predictive factors for these types of events.

Prediction of vertebral fractures

Factors predicting collapse of thoracic or lumbar vertebrae within the first 12 months were related to pre-

Table 3. Parameters with significant predictive value for vertebral fractures occurring later than 1 year after diagnosis in 28 multiple myeloma patients treated with standard chemotherapy.

Log-rank test	*p*
Baseline data	
Vertebral fracture score	0.003
Time-dependent variables:	
Whole body BMD, % changes	0.04
Urinary Ntx, nmol/mmol creat	0.014
Serum ICTP, μg/L*	0.025
Serum osteocalcin, μg/L*	0.029

**four patients with creatinine clearances less than 50 ml/min were excluded.*

Table 4. Parameters with significant predictive value for progressive osteolysis in 28 newly diagnosed multiple myeloma patients treated with standard chemotherapy.

Log rank test	*p*
Baseline data	
D&S stage III, categorical	0.022
Osteolysis score	0.011
Time-dependent variables:	
Serum ICTP, μg/L*	0.001
Urinary Ntx, nmol/mmol creat	0.02
Serum osteocalcin, μg/L*	0.007
Serum PINP, μg/L	0.01
BMD whole body, % changes	0.021

**four patients with creatinine clearance less than 50 ml/min were excluded; D&S, Durie& Salmon.*

therapy characteristics (Table 2). Elevated baseline ICTP and NTx had prognostic value, but as time-dependent variables these markers did not give significant information. Pre-therapy lumbar spine rarefaction, extensive bone disease, and high β2microglobulin were also predictive of early fractures. Patients who experienced early vertebral fractures had lumbar osteopenia at diagnosis, in contrast to patients who did not have early fractures (lumbar spine BMD Z-scores (mean ± SEM): −1.39 ± 0.34 vs. 0.15 ± 0.33; $p < 0.01$). Vertebral fracture score and β2microglobulin entered the multivariate analysis, and these two parameters provided the best model for predicting early vertebral collapse (Table 2).

Eight of 20 observed patients had at least one vertebral fracture episode after one year of treatment. These late vertebral collapses were significantly predicted by the factors in Table 3. Time-dependent elevated levels of ICTP and NTx, and loss of whole body BMD were predictive of late fractures.

Prediction of osteolytic events

Table 4 summarizes the factors predictive for progression of lytic bone lesions. The biochemical markers of bone resorption were superior to other factors for predicting progressive osteolysis.

Discussion

Serum ICTP and urinary NTx significantly predicted progressive bone disease, and thus also disease progression according to EBMT criteria.[34] From a clinical point of view, it is important to know whether these parameters provide information for daily practice by using upper normal reference values as cut-offs for identifying a patient with an increased risk of developing progressive bone disease. This information could allow re-institution or alteration of chemotherapy, and institution or intensification of bisphosphonate treatment. Thus, another important observation of this study was that the M component in itself did not provide information concerning bone degradative activity in all patients. In spite of a declining or stable M component, the myeloma clone can be metabolically and/or proliferatively active within the bone marrow compartment.

In our cohort of patients, ICTP and NTx were superior to the M component in predicting osteolytic events. Progression of bone osteolysis was preceded by elevated ICTP in almost all patients, whereas NTx levels were not consistently elevated. All patients with elevated ICTP and increased NTx over time showed progression of bone lesions within the following months. These observations indicate that ICTP and NTx are useful parameters for monitoring ongoing bone damage. However, it should be emphasized that our cohort of patients is rather small, and therefore, our findings need confirmation by a larger study. Moreover, all our patients were treated with MP as first line therapy, and our data and conclusions may not be applicable to patients treated with VAD or high-dose chemotherapy.

Unlike the markers of bone resorption, the markers of bone formation were within the normal range in almost all patients. This finding is in accordance with earlier studies and supports the general assumption that osteoblastic function is inhibited in MM.[1] However, our observation that levels of markers of bone formation were higher in patients with progressive osteolysis than in patients with no progression is somewhat in contrast to what might be expected, as is our finding that time-dependent increasing levels of osteocalcin and PINP significantly predicted osteolytic events. Theoretically, at least two factors might explain our observations. Firstly, trabecular microfrac-

tures in different areas of the skeleton may be a consequence of increased bone degradative activity and precede development of lytic lesions, and these microfractures might induce healing by formation of new bone matrix by osteoblasts. Secondly, it is possible that pathological bone remodeling may occur at different steps at the same time in varying parts of the skeleton. Locally, high concentrations of myeloma cells in micro- or macro-myelomas may produce a strong osteoclast-stimulating response and concomitant osteoblast inhibition in the vicinity,[35,36] hereby producing local osteolysis, whereas the bone remodeling process in other less myeloma infiltrated areas of the bone marrow may be balanced at the same time.

Indeed, our densitometry data suggest that the bone involvement can evolve heterogeneously in different parts of the skeleton. Thus, we observed a variable BMD response to treatment in many patients. For instance, in a single patient, the lumbar spine BMD increased by nearly 10% over 12 months, whereas the hip BMD declined by 10% over the same period. The opposite pattern was observed in other patients, although the most common observation was increasing lumbar BMD, while hip BMD declined (Figure 4). Due to evolution of the myeloma clone,[37] two or more sub-clones may proliferate simultaneously in a given patient, one being chemosensitive and the other being less responsive or resistant to treatment. This could explain a differential BMD response to treatment in different parts of the skeleton.

Vertebral collapses are common in MM. Many patients have early vertebral collapses within the first weeks or months after starting treatment. In our cohort patients with multiple vertebral collapses and a high serum β2microglobulin were particularly at risk. Furthermore, lumbar spine rarefaction at diagnosis was correlated with an increased risk of early fracture. A DEXA scan at diagnosis may be of value for assessing fracture risk, particularly in patients with no or few vertebral fractures.

Unlike early vertebral collapses, vertebral fractures occurring later in the course could be predicted by time-dependent parameters. Elevated NTx, ICTP, and declining whole body BMD over time are bad prognostic factors that theoretically can be affected by intensified chemotherapy and treatment with bisphosphonates. Surprisingly, we did not observe any predictive value of lumbar spine BMD changes. A possible explanation for this is the rather limited number of patients examined.

The sequential DEXA scans gave information on the dynamics of the bone disease in MM. Changes in local BMD were individual and did not always correspond to traditional criteria of response to treatment

An unexpected finding was that patients with no change in disease had better preserved whole body and hip BMD after one year than did patients with responsive disease. Patients with no change in disease, termed primary stable disease by some, may have lower metabolic and bone remodeling activity along with lower proliferative activity. It is a common observation that these patients often have a rather indolent course and often a good prognosis. However, it is somewhat unexpected that hip and whole body BMD of responders decrease more than in patients with no change disease. Nevertheless, the same observation was made in an earlier study by Mariette *et al.*[21] In line with our findings, these authors observed that patients with responsive disease had a 4.1% increase in lumbar spine BMD after 12 months but a concomitant 3% decrease in whole body BMD (-1.8% in our study). The authors suggest a redistribution of whole body calcium in responsive patients. Calcium might be actively resorbed from the appendicular skeleton and used for recalcification of the remodeling space of the axial skeleton from where myeloma cells disappear during treatment. The simultaneous decline in hip BMD observed by us, which the same authors also reported in another study,[20] could be explained by differences in the remodeling process in cancellous and cortical bone. However, we observed that patients with no change disease had increasing lumbar spine BMD without having declining whole body BMD. So, another factor within the biology of MM may be of importance.

Overall, lumbar spine BMD in patients with non-progressive disease increased by 2.6% after 1 year of treatment. This observation is in accordance with some earlier studies,[20-22] whereas another study showed a significant mean decline of 6.6%.[23] However, in this latter study by Diamond *et al.*[23] the patients examined had all been treated with pulse melphalan and prednisolone for 1 to 12 years prior to the study, so the authors did not have the opportunity to observe an early response to the start of treatment. By including newly diagnosed MM patients, Mariette *et al.*[21] found that patients with responsive disease had a 4.1% increase in their lumbar spine BMD after 12 months.

Diamond *et al.*[23] proposed that steroids may induce declining BMD over time. They found that the cumulative dosage of administered prednisolone was a significant predictor for the loss of lumbar spine BMD, as observed in their study. In the long term, the use of prednisolone might very well influence the bone remodeling process and contribute to bone loss but, as stated above, we did not observe lumbar spine bone loss during cyclic prednisolone treatment during the first 2 years of follow-up.

In conclusion, our data suggest that assays for serum ICTP and urinary NTx may be useful in clinical practice

for monitoring ongoing bone damage in myeloma patients, and thus be useful in predicting disease progression. Elevated levels of ICTP and NTx indicate insufficient disease control and increased risk of progressive bone events. Owing to a higher sensitivity, the ICTP assay seems preferable. However, the ICTP assay has a limited value in patients with impaired renal function, and in these patients the urinary NTx assay should be preferred. Patients with smoldering myeloma and MM patients with normal radiographs form subgroups in which the assays might be particularly useful. Elevated levels of the markers of bone resorption may suggest the opportuneness of initiating anti-myeloma therapy in patients with smoldering myeloma and bisphosphonate treatment in patients without lytic bone disease. Our study did not include patients treated with prophylactic bisphosphonates, and the study cannot answer whether the assays could be used for individualized dose titration of bisphosphonates in MM patients. Further studies on the use of these markers in multiple myeloma are warranted.

NA, KB, JLN, LH designed the trial and analyzed the results. NA drafted the paper. NA, KB, EFE, LH performed all the biochemical analyses. KB, EFE performed DEXA scans. JEK analyzed the radiographs. NA, KB, JEK, EFE, JLN, LH revised the paper and gave final approval of the version to be submitted. Nurse Hanne Lysgaard is very much appreciated for her skilful assistance. Kirsten Hald and Vibeke E. Jensen are kindly thanked for excellent technical assistance.

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