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Enzymes and Protein Markers

Biochemical Markers of Bone Formation in Patients with Plasma Cell Dyscrasias and Benign Osteoporosis

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Background: **Myeloma-induced bone loss is related to an uncoupling of bone formation and bone resorption. The aim of the present study was to assess the potential clinical value of biochemical markers of bone formation in the work up of patients with plasma cell dyscrasias.**
Methods: **Serum total alkaline phosphatase, bone-specific alkaline phosphatase (BAP), and osteocalcin (OC) were measured in 43 patients with newly diagnosed multiple myeloma (MM), in 40 patients with monoclonal gammopathy of undetermined significance (MGUS), in 40 patients with untreated benign vertebral osteoporosis (OPO), and in 48 healthy adults.**
Results: **In MM and MGUS patients, serum BAP, but not serum OC, was lower than in healthy controls ($P < 0.05$). Serum OC was higher in patients with OPO than in healthy controls ($P < 0.05$). The strongest associations between markers were found in OPO patients and in healthy adults. MM patients with early-stage disease or without detectable osteolysis had decreased serum BAP values ($P < 0.05$). Serum OC was higher in MM patients with stage III disease ($P < 0.05$) than in healthy controls. MM patients with OPO-like bone involvement had lower BAP values than sex- and age-matched MGUS patients with OPO-like bone involvement and patients with benign OPO ($P < 0.05$).**
Conclusions: **In patients with plasma cell dyscrasias, serum BAP, rather than serum OC, appears to reflect a suppressed bone formation rate and may be helpful in the differentiation between benign and myeloma-induced OPO. However, the overall clinical use of biochemical markers of bone formation in patients with plasma cell dyscrasia appears limited.**

Common features of overt multiple myeloma (MM)[3] are the circumscribed destruction of bone (i.e., osteolysis) or diffuse osteoporosis (OPO)-like changes *(1–3)*. Analyses of bone biopsies from MM patients revealed that the development of lytic bone lesions is not only related to increased osseous breakdown, but also to the uncoupling of bone formation and bone resorption *(4)*. Thus, during progression of MM-induced bone destruction, concentrations of histomorphometric markers of bone formation decrease, whereas bone resorption remains abnormally high *(4–6)*.

The evaluation of the degree of bone involvement in plasma cell dyscrasias appears to be of particular importance for clinical guidance *(7)*, but it is limited by the invasiveness of the currently available standard method (quantitative bone histology). We and others have previously demonstrated that biochemical markers of bone resorption are markedly higher in MM patients than in patients with monoclonal gammopathy of undetermined significance (MGUS) or in patients with benign OPO *(8–12)*. These findings indicate a significant clinical value of these indices in the noninvasive evaluation of myeloma-induced bone disease *(8–12)*. However, some reports based on histomorphometric *(4, 5)* and biochemical *(13)* analyses indicate that the inhibition of bone formation is of equal or even greater importance for myeloma-associ-

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[3] Nonstandard abbreviations: MM, multiple myeloma; OPO, osteoporosis; MGUS, monoclonal gammopathy of undetermined significance; OC, osteocalcin; BAP, bone-specific alkaline phosphatase; TAP, total alkaline phosphatase; BMD, bone mineral density; and DPD, deoxypyridinoline.

ated bone destruction than the increase in bone resorption.

Both serum osteocalcin (OC) and the serum activity of the bone-specific isoenzyme of alkaline phosphatase (BAP) are considered specific biochemical indices of bone formation *(14)*. Alkaline phosphatase is a ubiquitously distributed enzyme that is produced by a variety of cells from different tissues. However, >95% of the total serum activity of alkaline phosphatase (TAP) is derived from liver cells and osteoblasts *(15)*. Therefore, in subjects with healthy liver function, serum TAP can be a useful index of bone formation *(14)*. Several studies have indicated that the quantification of BAP in serum may provide a more specific index of bone formation *(16–19)*. With the development of immunoassays using specific antibodies against human BAP *(19–21)*, some of the limitations of the older assays, e.g., the somewhat cumbersome and labor-intensive assay formats, the relatively high assay variability, and the cross-reactivity with the liver isoenzyme, have been partly overcome. Although the immunoassays still show some cross-reactivity with the liver isoenzyme, clinical data seem to point toward an improved diagnostic validity of these new assays with regard to bone diseases and their therapeutic monitoring *(19–21)*. OC, or bone GLA-protein, is one of the major components of the noncollagenous bone matrix. The 5-kDa glycoprotein, which binds hydroxyapatite, is vitamin K and vitamin D dependent, and is almost exclusively osteoblast derived. OC is thought to play a role in the organization of the extracellular bone matrix. The largest part of newly synthesized OC is incorporated into the matrix *(22)*. A smaller fraction is released into the circulation where it can be quantified by various immunoassays *(22, 23)*.

Several investigations have studied biochemical markers of bone formation in patients with MM *(13, 24–27)*. However, the results varied considerably between the studies, and a systematic analysis of the markers with regard to the tumor stage and the differentiation between benign and myeloma-induced bone disease has not been reported. Therefore, the present study was carried out to determine the clinical usefulness of noninvasive measures of bone formation, namely serum BAP and OC as two of the most specific indices using two new immunoassays, in patients with plasma cell dyscrasias. Age- and sex-matched healthy adults and patients with benign vertebral OPO were included as control groups.

Patients and Methods

STUDY DESIGN

The present study was performed as a cross-sectional investigation to compare serum concentrations of biochemical markers of bone formation in patients with MM, MGUS, and OPO and healthy sex- and age-matched controls. We included 170 individuals, and all of the participants were studied at the time of primary diagnosis (Table 1). In all subjects, a complete history and physical exam were taken at the time of sample collection. Before study entry, none of the patients had been treated with chemotherapy, radiotherapy, glucocorticoids, or bisphosphonates. Written informed consent was obtained from all participants before the collection of blood samples. The results of tests evaluated in the study were not used in arriving at the patients' diagnoses. The study was approved by the local ethics committee and performed in accordance with the Declaration of Helsinki.

SELECTION OF PATIENTS WITH MM AND MGUS

Forty-three consecutive patients with overt MM and 40 patients with MGUS were included in the present study (Table 1). Five patients in the MGUS group fulfilled the diagnostic criteria for smoldering myeloma. Like patients with MGUS, they did not suffer from anemia, renal failure, or osteolytic bone lesions, but they did present with a higher plasma cell content (>10% and <30% of nucleated cells) in the bone marrow. All patients with plasma cell dyscrasias were characterized by the presence

Table 1. Population characteristics at baseline [median (range)].

	Healthy adults (n = 48)	MM (n = 43)	MGUS (n = 40)	Benign OPO (n = 40)
Sex,[a] M/F	17/31	14/29	16/24	15/25
Age, years	65 (33–84)	64 (28–87)	64 (40–86)	70 (45–83)
No bone disease[a,b]	48	7	27	
Osteoporosis-like bone involvement[a,b]		23	13	40
Osteolysis[a,b]		29		
Vertebral fractures[a,b]		19	7	40
S-Cr,[c] mg/L	9.0 (6.0–12.0)	10 (4.1–14)	9.0 (7.0–14)	10 (5.0–14)
S-Ca, mmol/L	2.42 (2.32–2.62)	2.18 (1.58–3.29)	2.36 (2.02–3.13)	2.43 (2.26–2.6)
β2, g/L	ND	3.75 (1.4–16.3)	1.75 (0.9–4.0)	1.6 (1.0–3.9)
t-Prot, g/L	71 (62–78)	87 (57–143)	78 (62–97)	73 (62–83)
PCC, %	ND	40 (0–95)	5 (0–20)	ND

[a] Number of patients.

[b] Data refer to the evaluation of bone disease by plain radiographs.

[c] S-Cr, serum creatinine; S-Ca, serum calcium, corrected for whole protein content; β2, β_2-microglobulin in serum; t-Prot, total protein; PCC, plasma cell content of the bone marrow; ND, not done.

of a monoclonal protein initially detected by serum or urine electrophoresis. Median serum concentrations of the monoclonal protein were 30.0 g/L (range, 10.6–91.9 g/L) in MM patients and 14.8 g/L (range, 5.7–38.3 g/L) in MGUS patients.

All patients underwent conventional x-ray examination of the skull, spine, pelvis, and painful regions outside the axial skeleton. Bone biopsies were performed with the Yamshidi needle after collection of blood and urine samples for subsequent biochemical analyses.

The differential diagnosis between MM and MGUS was based on the criteria of Salmon and Cassady *(1)*, and the staging of MM was based on the system of Durie and Salmon *(28)*. At the time of diagnosis, 9 patients with MM presented with stage I, 11 patients with stage II, and 24 patients with stage III disease. None of the MM patients included in the present study had serum creatinine concentrations >135 μmol/L. For further analyses, MM patients were subclassified according to bone disease: (*a*) no bone disease (n = 7); (*b*) OPO-like bone involvement with or without pathological fractures (n = 7); and (*c*) osteolysis with or without pathological fractures (n = 29).

In the MGUS group, 13 patients (8 women and 5 men; median age, 75 years) presented with radiologic evidence of osteoporotic bone loss *(29)* (Table 1). Vertebral fractures were found in six women, as well as in a 55-year-old man whose radiographs showed no signs of osteoporotic bone loss. The radiographically determined vertebral deformities in the six female patients appeared typical for benign OPO, although malignant bone involvement could not be completely excluded. In the male patient, the change in the shape of the vertebral body was explained as the result of an old trauma.

selection of patients with opo

Forty patients with newly diagnosed and untreated OPO were included. Of the 25 women in this group, all were postmenopausal with a median duration of menopause of 17 years (range, 2–41 years). The diagnosis of OPO was based on the presence of at least one vertebral fracture (wedge, compression, or biconcave) on conventional radiographs that was not attributable to adequate spinal trauma, and a bone mineral density (BMD) at the femoral neck, total hip, or lumbar spine (determined by dual x-ray absorptiometry) <2.5 SD of the age- and sex-matched mean. Mean femoral neck BMD values in these patients were 0.605 (± 0.084) g/cm^2 for women and 0.655 (± 0.081) g/cm^2 for men, mean total hip BMD values were 0.685 (± 0.134) g/cm^2 for women and 0.808 (± 0.112) g/cm^2 for men, and mean lumbar spine (L2–4) BMD values were 0.747 (± 0.149) g/cm^2 for women and 0.79 (± 0.114) g/cm^2 for men. None of the subjects in this group had a history of malignant disease, nor were there any signs of plasma cell dyscrasia or secondary OPO. At the time of study enrollment, all patients had healthy renal and hepatic function, and none was given any medication known to interfere with bone turnover, including bisphosphonates, glucocorticoids, hormone replacement therapy, osteotropic vitamins, or calcium supplementation.

selection of healthy controls

The control group comprised an ambulatory population of 48 normocalcemic adults without any evidence of skeletal or nonskeletal disease. All women in this group were postmenopausal with a median duration of menopause of 13 years (range, 2–35 years). All subjects had conventional radiographs of the lumbar and thoracic spine (Multiplanimat; Siemens). Femoral neck, total hip, and lumbar spine BMD values were determined by dual x-ray absorptiometry (QDR 1000; Hologic). Subjects with vertebral fractures, a BMD <2 SD of the age- and sex-matched mean, substantial degenerative disease of the spine, or abnormal laboratory results were excluded. Mean femoral neck BMD values in the controls were 0.713 (± 0.116) g/cm^2 for women and 0.818 (± 0.137) g/cm^2 for men, mean total hip BMD values were 0.851 (± 0.136) g/cm^2 for women and 0.988 (± 0.155) g/cm^2 for men, and mean lumbar spine (L2–4) BMD values were 0.919 (± 0.178) g/cm^2 for women and 1.048 (± 0.192) g/cm^2 for men. None of the controls was taking any medication known to affect bone metabolism, such as bisphosphonates, glucocorticoids, hormone replacement therapy, osteotropic vitamins, or calcium supplements.

laboratory values

Blood and urine samples were obtained simultaneously between 0800 and 1100 with subjects having had their usual breakfast. Venous blood was collected in Vacutainer Tubes without additive, allowed to clot for 30–45 min at room temperature, and centrifuged at 1000*g* for 10 min. Serum aliquots were stored at −80 °C. Urine samples were spot urines and were stored at −30 °C until analysis. All analyses were performed without knowledge of the diagnoses of the patients.

Routine biochemical analyses were performed in all participants, including red and white blood cell counts, serum calcium, serum and urinary creatinine, serum γ-glutamyl transpeptidase, albumin, total protein, β_2-microglobulin, and serum and urine electrophoresis. No dietary restrictions were applied. Serum calcium was corrected for whole protein content according to the method of Husdan et al. *(30)*.

Serum TAP activity was determined by an automated colorimetric assay with a BM/Hitachi System 704 analyzer (Boehringer Mannheim) at 37 °C with *p*-nitrophenyl phosphate as substrate *(31)*. All values were recalculated to a temperature of 25 °C. Intra- and interassay CVs were <5%.

An enzyme immunoassay (Alkphase-B; Metra Biosystems) was used to measure BAP. Characteristics of the assay have been published elsewhere *(21)*. In short, the microtiter plate format immunoassay used a plate-coated monoclonal anti-BAP capture antibody, and the activity

of the captured enzyme was detected with *p*-nitrophenyl phosphate as substrate. The reported cross-reactivities of the monoclonal antibody with isoenzymes of the alkaline phosphatase produced in the liver, intestine, and placenta are 5%, 0.4%, and 0%, respectively. Intra- and interassay CVs were 3.2–3.5% and 6.2–7.9%, respectively.

Serum intact OC was measured by an immunoradiometric assay (Elsa-Osteo; CIS Bio International). The assay is based on the application of two highly specific antibodies against human OC *(23)*. Intra- and interassay CVs were 3.0% and 6.5%, respectively.

Total urinary deoxypyridinoline (DPD) was included as a reference marker of bone resorption and was determined by HPLC after acid hydrolysis of the urine *(32)*. After partition chromatography on a CF1 cellulose column, DPD in samples and external standards was separated by reversed-phase ion-paired HPLC and quantified by fluorometry. The overall reproducibility of this assay was 8–12%, including the partitioning step. Values were expressed relative to urinary creatinine concentrations.

STATISTICAL ANALYSIS

The SAS software package was used for statistical analysis. To illustrate the distribution of marker values in the respective disease groups, descriptive statistics are presented as median and range unless otherwise stated. Simple Pearson regression analyses were performed to assess the strength of association between markers. Group differences were determined using analysis of variance on ranks or the Wilcoxon rank-sum test for nonparametric variables. All statistical tests were two-tailed, and $P < 0.05$ was considered statistically significant. To correct for multiple testing, *P* values were adjusted according to the Bonferroni correction (significance/number of tests).

Results

BIOCHEMICAL MARKERS OF BONE FORMATION IN HEALTHY CONTROLS AND IN PATIENTS WITH MM, MGUS, OR OPO

As shown in Table 2, median concentrations of serum BAP activity were significantly lower in patients with MM and patients with MGUS than in healthy controls ($P < 0.05$, respectively). In contrast, serum OC concentrations were significantly higher in patients with OPO compared with the control group ($P < 0.05$), but did not show any significant changes in the MM or the MGUS group. No significant differences in median serum concentrations of TAP were found among the three groups of patients and healthy controls (Table 2 and Fig. 1). For comparison, urinary DPD as a marker of bone resorption was included in the analysis and showed the highest values in MM patients ($P < 0.001$ vs healthy controls). Patients with MGUS and OPO had significantly higher median concentrations of DPD compared with healthy controls ($P < 0.05$ and $P < 0.01$, respectively) and significantly lower values compared with MM patients ($P < 0.01$; Table 2).

Simple Pearson correlation analyses revealed the strongest positive associations between serum TAP, BAP, and OC in the control group and in patients with OPO. In patients with MGUS, the strength of association between markers was weaker with a statistically significant correlation coefficient for serum TAP vs BAP only. In patients with MM, only the correlation between serum TAP and BAP reached statistical significance (Table 3). No significant correlations were found between urinary DPD and any of the bone formation markers in any of the groups.

In patients with MM, a weak but significant association was found between serum β_2-microglobulin and serum BAP ($r = 0.36$; $P < 0.05$). Otherwise, no significant correlations were found between any of the bone formation markers and routine laboratory values in any of the control or disease groups.

EFFECT OF TUMOR STAGE AND EXTENT OF BONE DISEASE ON BIOCHEMICAL MARKERS OF BONE FORMATION

When MM patients were stratified according to the staging system of Durie and Salmon *(28)*, patients with stage I and II disease had significantly lower serum concentrations of BAP than healthy controls ($P < 0.05$). No statistically significant differences in serum BAP concentrations were found between stage III and the early stages or healthy controls. Median serum OC concentrations were higher in patients with stage III disease compared with controls ($P < 0.05$), but did not differ significantly among the stages. Serum concentrations of TAP did not differ among the stages and healthy controls (Fig. 1).

Stratification of patients with MM, according to the extent of neoplastic bone involvement as judged from plain radiographs, revealed a distinct pattern for the three bone formation markers (Fig. 2). Serum TAP concentrations were significantly higher in patients with overt osteolytic lesions compared with healthy controls, MM

Table 2. Biochemical markers of bone formation in healthy controls and the disease groups [median (range)].

	Healthy controls (n = 48)	MM (n = 43)	MGUS (n = 40)	Benign OPO (n = 40)
TAP, U/L	101 (63–168)	113 (42–292)	107 (64–250)	108 (65–245)
BAP, U/L	21.4 (11.1–41.2)	17.7 (6.1–45.4)[a]	16.3 (6.8–46.6)[a]	18.5 (8.5–43.7)
OC, μg/L	18.5 (8.4–46.0)	20.0 (6.1–65.0)	19.2 (5.6–46.0)	22.6 (12.2–45.0)[a]
DPD, nmol/mmol of creatinine	5.3 (2.2–17.7)	12.0[b] (3.9–314)	6.1[a,d] (3.0–21.8)	7.9[b,c,d] (2.7–21.5)

[a–c] Compared with healthy controls: [a] $P < 0.05$; [b] $P < 0.001$; [c] $P < 0.01$.
[d] $P < 0.01$ vs MM.

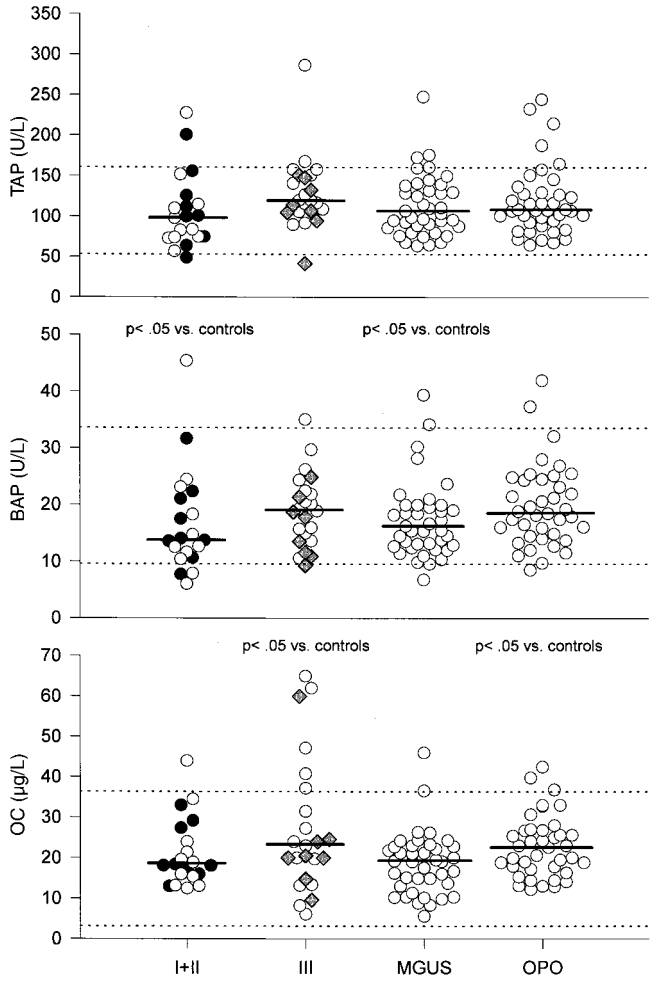

Fig. 1. Biochemical markers of bone formation in patients with MM stratified according to disease stage, MGUS, and benign OPO.

Solid lines represent the respective medians. *Areas between the dotted lines* represent the reference intervals calculated as the mean ± 2 SD of healthy controls. *I+II*, MM patients with stage I (●) and stage II (○) disease; *III*, normocalcemic (○) and hypercalcemic (◊) MM patients with stage III disease; *OPO*, patients with benign OPO.

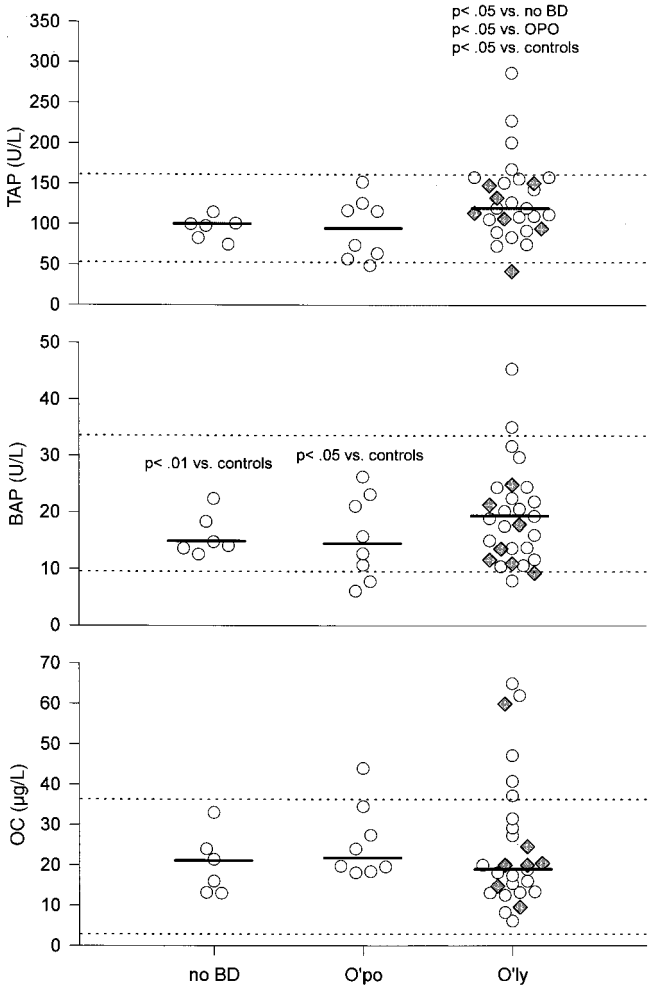

Fig. 2. Biochemical markers of bone formation in patients with MM stratified according to type of bone disease.

Solid lines represent the respective medians. *Areas between the dotted lines* represent the reference intervals as calculated as the mean ± 2 SD of healthy controls. *no BD*, MM patients without detectable bone involvement; *O'po*, MM patients with OPO-like bone involvement; *O'ly*, MM patients with overt osteolysis; ○, normocalcemic MM patients; ◊, hypercalcemic MM patients.

patients with no detectable bone disease, and MM patients with OPO-like changes ($P < 0.05$). In contrast, serum BAP concentrations were significantly lower in MM patients with no detectable bone disease and with OPO-like bone involvement compared with healthy controls ($P < 0.01$ and $P < 0.05$, respectively), but did not show significant alterations in the group of patients with overt osteolytic lesions. Serum concentrations of OC did not differ among the groups (Fig. 2).

Table 3. Simple Pearson correlations between biochemical markers of bone formation in healthy controls and the disease groups.

	Healthy controls (n = 48)	MM (n = 43)	MGUS (n = 40)	Benign OPO (n = 40)
TAP vs BAP	$r = 0.76$[a]	$r = 0.46$[b]	$r = 0.72$[a]	$r = 0.90$[a]
TAP vs OC	$r = 0.58$[b]	NS[c]	NS	$r = 0.65$[a]
BAP vs OC	$r = 0.63$[a]	NS	NS	$r = 0.69$[a]

[a] $P < 0.01$.
[b] $P < 0.05$.
[c] NS, not significant.

The differentiation between benign and neoplastic OPO-like changes is of particular clinical interest. Therefore, we compared bone formation markers from the seven MM patients with OPO-like bone involvement and without osteolytic lesions with seven sex- and-age matched patients with benign OPO and with seven sex- and age-matched patients with MGUS and OPO-like bone disease. As shown in Fig. 3, MM patients with OPO-like bone involvement had on average lower serum concentrations of TAP, BAP, and OC compared with patients with benign OPO. However, this difference was statistically significant for serum BAP only ($P < 0.05$). None of the markers differed significantly when the results from

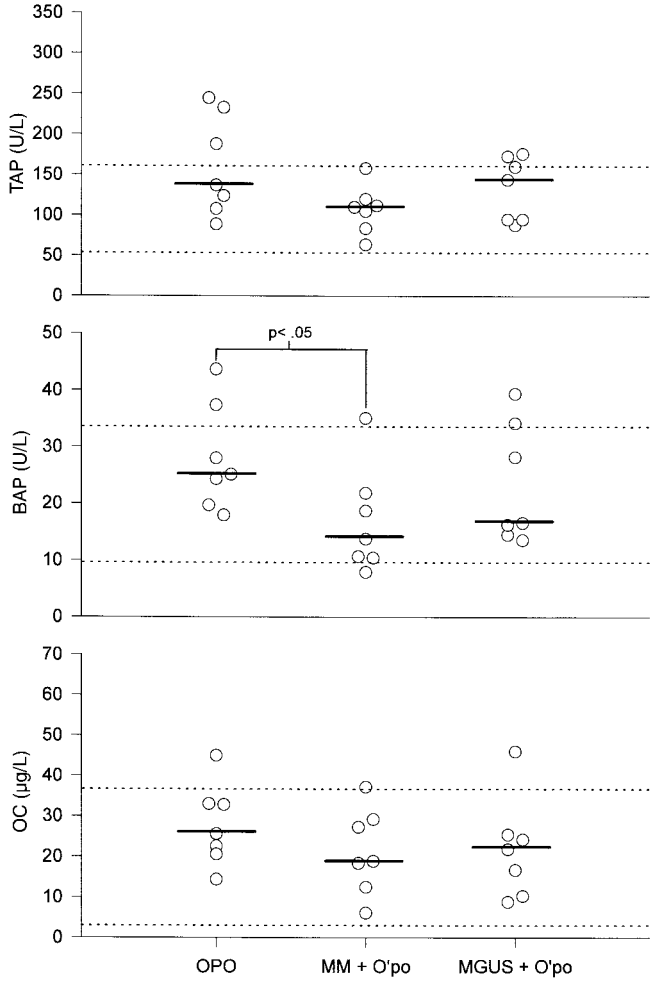

Fig. 3. Biochemical markers of bone formation in patients with benign OPO and in MM or MGUS patients with OPO-like bone involvement.

Solid lines represent the respective medians. *Areas between the dotted lines* represent the reference intervals as calculated as the mean ± 2 SD of healthy controls. *OPO*, patients with benign OPO; *MM + O'po*, MM patients with OPO-like bone involvement; *MGUS + O'po*, MGUS patients with OPO-like bone involvement.

the MGUS group were compared with either of the other two groups (Fig. 3).

Discussion

Progressive MM is associated with both a suppression of bone formation and a stimulation of bone resorption as shown by histomorphometric studies *(4–6)*. This uncoupling of bone turnover is thought to be responsible for the development of osteolytic lesions. We and others have previously demonstrated the clinical value of biochemical markers of bone resorption in the noninvasive evaluation of malignant bone disease in MM patients *(8–12)*. However, some reports based on histomorphometric analyses have indicated that the inhibition of bone formation is of equal or even greater importance for myeloma-associated bone destruction than the increase in bone resorption *(4–6)*. Therefore, the present investigation focused on noninvasive measures of bone formation as potentially helpful diagnostic tools in patients with plasma cell dyscrasias. We found a dissociation of serum concentrations of the biochemical indices studied: serum BAP was reduced in patients with MM and MGUS, whereas serum OC and serum TAP did not show any corresponding changes. Moreover, patients with myeloma-induced OPO seemed characterized by suppressed serum BAP activity compared with patients with benign OPO.

Both serum BAP and OC are considered specific biochemical markers of bone formation *(14)*. However, bone formation is a complex process, and the two markers differ largely with respect to process specificity, origin, and function *(14, 33)*. It is therefore not surprising that the value of either BAP or OC as the bone formation marker of choice varies considerably with regard to the specific clinical situation. For example, in Paget disease of bone, measurement of alkaline phosphatase activity (in most cases serum TAP rather than BAP, because of markedly lower costs and faster turnaround time) is the most practical marker in the evaluation of disease activity and therapeutic response to antiresorptive agents *(34)*. In contrast, although the reasons for this phenomenon are not elucidated, measurements of serum OC do not provide helpful clinical information in this group of patients *(34)*. The distinct pattern of results for serum BAP and OC in the present study was therefore not unexpected.

In the total group of MM patients, serum activity of BAP was significantly reduced, whereas OC concentrations were unchanged compared with healthy controls. In addition, we did not find any significant changes for serum TAP, indicating that it is indeed a less specific marker of osteoblast activity than BAP *(19–21)*. In our study, the uncoupling of bone turnover with reduced rates of bone formation in myeloma-induced bone disease seemed best reflected by serum BAP measurements. Somewhat surprising, however, is that our results showed that BAP activity was suppressed in the early stages of myeloma-induced bone disease and even in MM patients without any detectable bone involvement. Moreover, BAP concentrations were reduced in a considerable number of patients with MGUS. It has been shown in vitro that myeloma cells secrete certain, as yet unidentified, factors that inhibit osteoblast proliferation and function and may directly suppress alkaline phosphatase expression in osteoblasts *(35, 36)*. Direct inhibition may well explain the reduced concentrations of serum BAP activity in the MM patients in our study. It is conceivable that these factors may lead to reduced BAP activity even in early myeloma stages or monoclonal gammopathies without malign characteristics, although the total number of osteoblasts is increased in these situations *(5)*.

In contrast to the results for serum BAP, serum OC did not show significant changes with regard to myeloma-induced bone disease, and serum concentrations were rather increased in late-stage disease. These findings are also in contrast to reports based on histomorphometric

(4–6) and biochemical *(13)* analyses in which the suppression of bone formation and OC concentrations was associated with the later stages of MM disease. The discrepant findings for serum OC in our study compared with a report by Bataille et al. *(13)* may be explained by differences in the selected study population or by differences in assay characteristics *(22, 23)*. Serum OC concentrations are determined by several confounding factors. For example, although OC is produced by osteoblasts, it is released during bone matrix mineralization *(22)*. Thus, an inhibition of osteoblast proliferation by myeloma cells may not necessarily be accompanied by a simultaneously suppressed matrix mineralization. In addition, OC is a relatively unstable protein and is rapidly metabolized in the kidney after release into the circulation *(22)*. The utility of the large number of existing immunoassays for the quantification of OC in human serum is hampered by the instability of the protein and the modifications it undergoes after release from the bone matrix, with their impact on antibody recognition sites *(22)*. Moreover, renal failure is a common feature in advanced MM disease stages. Although MM patients with severely reduced renal function were excluded from the present study, the possibility of a moderate reduction in the glomerular filtration rate, which may produce falsely increased serum OC concentrations, cannot be completely ruled out. Furthermore, in vitro studies have provided evidence that, at least in some patients, OC may be synthesized by the myeloma cells themselves. For example, a myeloma cell line (NCI-H929) has been shown to express and secrete OC *(37)*. It is conceivable that ectopic OC production in some patients may have influenced the results of the present study.

In advanced tumor stages, suppressed bone formation is usually evident by histomorphometry *(4–6)*. However, in these situations, other systemic factors, including progressive renal failure, compensatory increased bone formation at skeletal sites that show no signs of neoplastic bone involvement, reduced mobility, or other secondary diseases are likely to influence biochemical measurements and lead to falsely increased serum concentrations of bone formation markers. Overall, these difficulties severely limit the clinical usefulness of both BAP and OC in patients with progressive MM. As demonstrated previously in the same population and in several other studies, measurements of biochemical markers of bone resorption, namely collagen degradation products *(8, 9)* and bone sialoprotein *(12)*, appear to have superior clinical value in these cases. The better performance of bone resorption indices may be attributable to the fact that the stage-specific increase in bone resorption is more clear-cut than the suppression of bone formation, and possible cofactors may intensify rather than diminish changes in resorption marker concentrations.

One interesting subanalysis in the present study was the comparison of markers of bone formation in patients with benign OPO and OPO-like changes in patients with MM or MGUS. In general, OPO is a very heterogeneous disease and characterized, in the majority of patients, by only mildly disturbed bone turnover at the time of diagnosis *(38, 39)*. In the present study, this was reflected by slightly increased OC concentrations and unchanged BAP activity in the OPO group. However, in the discrimination between benign and MM-associated OPO, the inclusion of a biochemical marker of bone formation may provide some important additional information. We found significantly reduced serum BAP concentrations in MM patients with OPO-like changes compared with age- and sex-matched patients with benign OPO. Although larger numbers of patients are required, suppressed markers of bone formation, especially serum BAP, may help to identify an underlying malign process in certain cases with OPO.

In conclusion, because of the results of the present study, we cannot recommend routine biochemical measurements of bone formation in patients with plasma cell dyscrasias at this point. However, they may be helpful in selected clinical situations. Because of marker characteristics, the quantification of serum BAP appears superior to the applied serum OC assay in certain instances of myeloma-induced bone disease.

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