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# Biochemical markers of bone disease in asymptomatic early stage multiple myeloma. A study on their role in identifying high risk patients

ALESSANDRO CORSO, LUCA ARCAINI, SILVIA MANGIACAVALLI, CESARE ASTORI, ESTER ORLANDI, ANGELA LORENZI, FRANCESCO PASSAMONTI, CATHERINE KLERSY*, CRISTIANA PASCUTTO, ATTILIA CANEVARI-SCIORATI, MARIO LAZZARINO

Institute of Hematology, University of Pavia,
*Biometric Unit-Scientific Direction,
Istituto Scientifico Policlinico San Matteo, Pavia, Italy

Correspondence: Alessandro Corso, M.D., Division of Hematology, Policlinico San Matteo, 27100 Pavia, Italy. Fax: international +39-0382-502250 - E-mail: a.corso@smatteo.pv.it

*Background and Objectives.* Skeletal involvement is typical of multiple myeloma (MM) and its occurrence increases with the progression of the disease. We performed a study to evaluate the clinical importance of osteocalcin (bone gla-protein, BGP) and bone alkaline phosphatase (b-AP) as indices of osteoblastic activity, and deoxypyridoline (DPD) as a marker of bone resorption.

*Design and Methods.* Fifty-two MM patients, 39 patients with monoclonal gammopathy of undetermined significance (MGUS), and 30 normal controls entered the study. Of the 52 MM patients, 10 showed lytic lesions at standard X-rays and 42 did not; 21 were untreated and 31 had been treated with chemotherapy (combined with bisphophonates in 15). Of these last, 12 had progressive disease and 19 were in plateau phase.

*Results.* DPD levels were higher in MM patients than in patients with MGUS or healthy controls ($p$=0.0001 and $p$=0.0008, respectively). No statistical differences were seen between patients with MGUS and healthy controls. BGP serum levels were significantly lower in MM patients than in MGUS patients ($p$=0.001) or healthy controls ($p$=0.001). b-AP was significantly higher in MGUS patients than in MM patients ($p$=0.04). Biochemical parameters were analyzed in a continuous fashion and after dichotomization into low and high values with respect to normal ones. Abnormal high values of DPD showed statistically significant correlations with presence of osteolysis ($p$=0.008), advanced stage ($p$=0.03) and abnormal $\beta_2$-microglobulin ($\beta_2$M) values ($p$=0.03), while DPD as a continuous variable correlated significantly only with the presence of osteolysis ($p$=0.02). In contrast, neither BGP nor b-AP showed statistical correlations with the presence of lytic lesions, or with other clinical or laboratory parameters. In 15 patients followed with serial controls, modifications of DPD levels reflected bone disease status well. Of the 42 patients without radiologic evidence of skeletal lesions, 15 had abnormal DPD values. Spinal magnetic resonance imaging (MRI) showed initial lytic lesions in 10 of them.

*Interpretation and Conclusions.* Biochemical markers of bone metabolism are useful in evaluating and monitoring skeletal involvement in MM patients. They may help clinicians to identify: 1) from among patients without radiologic evidence of lytic lesions, those who deserve more accurate radiologic examinations (namely MRI); 2) from among asymptomatic patients, and in association with spinal MRI, those patients at higher risk of progression who might benefit from early treatment.


Key words: myeloma bone disease, bone–specific alkaline phosphatase, osteocalcin, deoxypyridoline

Multiple myeloma is characterized by a clonal proliferation of plasma cells, typically affecting the structure of bone. In fact, nearly 75% of patients at diagnosis have skeletal involvement with bone pain, lytic lesions, or pathologic fractures, and almost all patients run into skeletal events during the history of their disease. An uncoupled process between osteoclastic and osteoblastic activities has been hypothesized as the pathogenetic mechanism of bone disease. The osteoclastic activity at the beginning of the disease is counterbalanced by osteoblastic reaction, but during the course of the disease overwhelms it, resulting in overt osseous breakdown.[1] Bisphosphonates, alone or in association with chemotherapy, have proved to be very effective in ameliorating skeletal lesions and in reducing the incidence of skeletal events.[2] However, it is sometimes difficult to establish the best time to start administering these drugs. Several studies, in fact, have demonstrated that even in asymptomatic early stage patients, without any clinical or radiologic evidence of skeletal disease, bone metabolism may be altered.[3] Furthermore, there is a recent tendency for increasing numbers of patients to come to the attention of the physician in early stages and for these patients the indications for starting chemotherapy are equivocal. On the other hand, the population of asymptomatic first stage MM patients is not homogeneous, since the time to progression of disease ranges from a few months to some years.[4] Moulopoulos *et al.* have demonstrated that,

among asymptomatic patients, those who showed abnormal MRI patterns of the spine had a higher probability of progression than those without any radiologic evidence of disease.[5] Thus, more accurate staging of bone disease is necessary in order to identify patients who deserve early treatment.[6] A complete standard skeletal radiograph is the routine approach in spite of its poor sensitivity. MRI can demonstrate minimal bone lesions in 50% of patients with no lytic lesions at X-rays, but its use as a screening test is limited by its cost. Several studies have demonstrated that urinary and serum markers of bone metabolism are good alternatives to radiologic screening tests because of their specificity and reliability in reflecting osteoblastic and osteoclastic activity.[3,7-15] Markers of bone resorption, peptide fragments or pyridinium, can be assayed in the serum[8-10,12-14,16] or in the urine.[10-13,14] Markers of bone formation are osteocalcin and bone specific alkaline-phosphatase. All these markers have been widely reported as good, sensitive tests for identifying skeletal involvement in multiple myeloma and, in some cases, even as prognostic factors for survival.[9,14] It is not known, however, how they should be used in routine clinical practice, whether they should be used as an alternative to or in combination with radiologic examinations, particularly for monitoring bone disease. To our knowledge, only one study[12] uses these biochemical markers in monitoring bone turnover in patients with multiple myeloma.

The aims of this study were: 1) to evaluate the usefulness of serum and urine markers of bone metabolism in identifying bone involvement in patients with multiple myeloma, particularly in the early stages; 2) to investigate the correlations between these markers and other clinical and laboratory parameters; 3) to determine their role in monitoring bone disease during the course of myeloma.

## Design and Methods

### Patients

Fifty-two patients with multiple myeloma and 39 with MGUS were examined. Patients were diagnosed according to the criteria published for MGUS and myeloma.[17]

The MM patients, 37 males and 15 females, had a median age of 58 years (range 31-82). Their characteristics, collected at time of examinations, are reported in Table 1. All patients were characterized by serum and urine electrophoresis. The types of M-component (MC) were: 34 patients IgG, 14 IgA and 4 light chain. Patients were staged according to Durie and Salmon as follows: 26 stage I, 9 stage II, and 17 stage III. Standard X-rays showed lytic lesions in 10 patients whereas 42 were negative. Of the 52 MM patients 21 were untreated, and 31 had been treated with chemotherapy (15 in combination with bisphophonates). Of these last, 12 showed progressive disease and 19 were in plateau phase.

Of 39 MGUS patients with a median age of 58 (31-77) years, 18 were males and 21 females. Thirty-five had IgG gammopathy, 3 IgA and 1 IgM. Thirty normal controls were included: 14 males and 16 females with a median age of 37 years (range 28-57). None had a history of serious disease, nor had any fractures. None was taking any drug affecting bone metabolism.

**Table 1. Clinical and laboratory characteristics of 52 MM patients.**

| | |
|---|---|
| No. of subjects | 52 |
| Sex (male/female) | 37/15 |
| Age | |
| Median | 58 |
| Range | 31-82 |
| Type of component | |
| IgG | 34 |
| IgA | 14 |
| Light chain | 4 |
| Durie And Salmon stage | |
| Stage I | 26 |
| Stage II | 9 |
| Stage III | 17 |
| Standard radiology | |
| Normal | 42 |
| Lytic lesions | 10 |
| Treatment | |
| Treated | 31 |
| Untreated | 21 |

### Biochemical assays

Serum and urine samples were all taken in the same morning, 4-6 weeks after the last chemotherapy or last administration of bisphosphonates. Blood specimens were collected on ice, separated within 1 hour and immediately frozen at –20°C. Second void urine, collected by 10.00 AM was collected on ice and rapidly processed and stored at –20°C.

Free deoxypyridinoline was measured by a competitive ELISA (Pyrilinks-DTM) obtained from Metra Biosystems (Mountain View, CA, USA). The deoxypyridinoline values were corrected for variations in urine concentration by dividing the deoxypyridinoline value (nM) by the creatinine value (nM) of each sample.

Serum bone-specific alkaline phosphatase was analyzed with a non-isotopic immunoassay (Alkphase-BR) from Metra Biosystems (Mountain View, CA, USA). The bone-specific alkaline phosphatase assay measures enzyme activity in serum with a high specificity. Bone alkaline phosphatase (b-AP) is bound by a monoclonal antibody and phosphatase activity is measured by adding *p*-nitrophenyl phosphate substrate.

Competitive immunoassays (Novocalcin®) from Metra Biosystems (Mountain View, CA, USA) were used to measure osteocalcin. The assay directed against intact human osteocalcin showed a high specificity.

### Statistical analysis

Descriptive statistics are reported as mean and standard deviation for continuous variables and frequencies for categorical variables. Values of BGP, DPD and b-AP were dichotomized according to their normal values. The associations of BGP, DPD and b-AP with the pres-

**Table 2. Comparison of values of DPD, BGP, and b-AP (mean ± S.D.).**

| | *DPD (nM/mM creat.)* | *BGP (ng/mL)* | *b-AP U/L* |
|---|---|---|---|
| Normal Controls | 3.95 ± 1.08 | 7.18 ± 1.79 | 20.56 ± 5.46 |
| MM | 6.21 ± 2.42 | 4.29 ± 2.73 | 16. 91 ± 6.68 |
| MGUS | 3.96 ± 1.04 | 6.8 ± 2.33 | 25.53 ± 8,84 |
| MM vs MGUS | *p*=0.0001 | *p*=0.001 | *p*=0.04 |
| MM vs Controls | *p*=0.0008 | *p*=0.001 | NS |
| MGUS vs Controls | NS | NS | NS |

ence of lytic lesions, phase, stage, $\beta_2$-microglobulin ($\beta_2$M) and plasmacytosis were assessed by means of logistic regression, when dichotomized values were considered. Their continuous values were compared across levels of osteolysis, phase or stage by means of the Kruskall-Wallis test or the Mann Whitney U test. Spearman's correlation coefficient was computed for associations with β2M and plasmacytosis. Finally, the BGP, DPD and b-AP values in MM and MGUS patients and controls were compared by means of the Kruskall-Wallis test and *post-hoc* Mann Whitney U test (with Bonferroni correction for multiple tests).

All tests were two-tailed; $p<0.05$ was taken to be statistically significant. Stata 6.0 software (StataCorp, College Station, TX, USA) was used for all computations.

## Results

Comparison of mean values of urinary concentration of deoxypyridinoline, and of serum levels of osteocalcin and alkaline phosphatase are reported in Table 2. As shown, DPD levels were higher in MM patients than in MGUS patients or healthy controls ($p$=0.0001 and $p$=0.0008, respectively). No statistical differences were present between MGUS patients and healthy controls. BGP values were significantly lower in MM patients than in those with MGUS ($p$=0.001) or healthy controls ($p$=0.001). b-AP was significantly higher in MGUS patients than in MM patients ($p$=0.04).

Biochemical parameters were analyzed in a continuous fashion and after dichotomization into low and high values with respect to normal ones. When analyzed as a continuous variable, DPD correlated significantly only with the presence of osteolysis ($p$=0.02). Abnormal high values of DPD showed statistically significant correlations with osteolysis ($p$=0.008), disease stage ($p$=0.03) and $\beta_2$M ($p$=0.03).

In contrast, both osteocalcin and bone alkaline phosphatase did not show statistical correlations with the presence of lytic lesions, nor with other clinical or laboratory parameters.

Fifteen of the 52 patients underwent four-monthly controls for a minimum of three evaluations. Five patients (group I) showed clinical and radiologic improvement after chemotherapy and pamidronate given in association, three (group II) the occurrence of lytic lesions, and seven patients (group III) stable disease. Median BGP and DPD levels of the three groups are reported in Figure 1. As shown, the behavior of the two parameters, in particular of DPD, reflected the bone disease status well. Since the skeletal events in group II patients did not occur in all patients at the same time, their median DPD and BGP values were considered only at the time of first evaluation and at occurrence of osteolysis. In one of the patients with worsening bone disease, lytic lesions were detected only by MRI study.

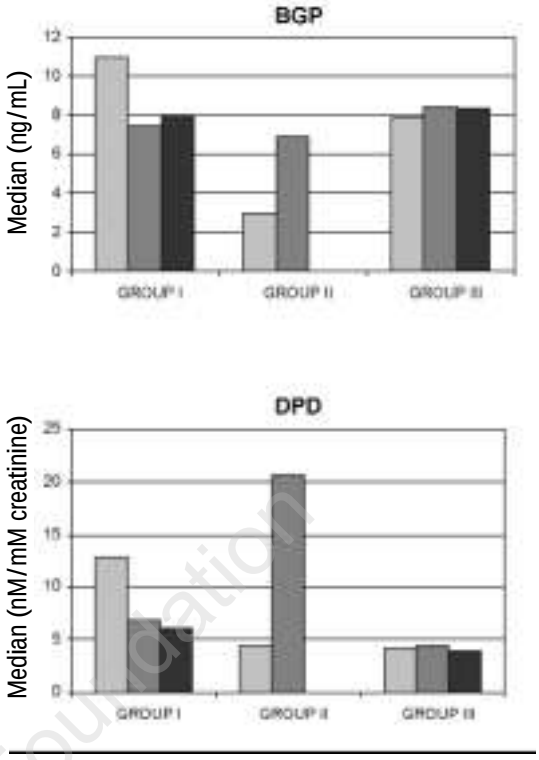


**Figure 1. Median BGP and DPD levels in 15 patients monitored for 1 year. Five patients (group I) showed clinical and radiological improvement after chemotherapy and pamidronate given in association, three (group II) developed lytic lesions, and seven patients (group III) had stable disease. The graph shows a good correlation between biochemical markers and bone disease status.**

Of the 42 patients without radiologic evidence of lytic lesions, 15 had abnormal DPD values. MRI study of the spine in 10 of them showed initial focal osteolytic lesions. Eleven of the 42 patients were at onset, with early stage disease. Of these, 7 had abnormal high values of DPD levels and 5 of them had initial osteolysis at MRI study.

One out of 5 patients with a normal MRI pattern of the spine, but abnormal values of biochemical markers, complained of bone pains that ameliorated with pamidronate treatment.

## Discussion

Multiple myeloma remains an incurable disease. Thus, the target of clinicians, at the moment, should be to prolong event-free survival and improve the patient's quality of life. Myeloma-related bone disease negatively affects the quality of life of patients, and is sometimes independent of the response to therapy. Traditionally, standard X-rays are used to evaluate bone involvement, even though this approach is known to be insufficient for identifying initial lytic lesions. MRI, on the other hand, has been claimed to be able to detect lytic lesions in 50% of patients with a negative radiologic exam, but is quite expensive and clearly cannot be proposed for routine screening in MM patients.

Biochemical markers have been widely studied, although data reported in the literature[3,7-15] do not yet give definitive and unequivocal information on either their prognostic relevance, or their use with respect to standard X-rays and MRI: together or as an alternative? Bataille *et al.*[3] reported low levels of BGP in MM patients with extensive lytic lesions and advanced disease. In our study, median levels of BGP in MM patients were significantly lower than those in MGUS patients and healthy controls. However, when statistical analysis was performed, BGP was not significantly correlated with the presence of osteolysis, disease stage, or other parameters of disease activity. Abildgaard *et al.*[10] demonstrated that serum bone-specific alkaline phosphatase (bAP) and C-terminal propeptide of procollagen I (PICP) were significantly correlated with bone disease, as evaluated by histomorphometric study. We did not find correlations with the clinical or laboratory findings of MM patients, but median b-AP levels were lower in MM patients than in MGUS patients.

In our study, the median levels of DPD were significantly higher in MM patients than in MGUS patients or healthy controls, as reported by others.[11, 13] Abnormal high DPD levels were strongly correlated with extent of bone involvement, advanced disease stage, and β–2microglobulin levels. This is consistent with the fact that bone disease has an important role in the Durie and Salmon staging, and that skeletal involvement is nearly constantly present in the advanced phase of the disease. This last is generally characterized by a high tumor burden and by a higher level of $\beta_2$M.

Fifteen patients had abnormal values of laboratory markers without radiologic evidence of lytic lesions. In 10 (66%) of them MRI study of the spine showed early bone involvement, and one of the remaining patients who complained of bone pain improved with the administration of bisphosphonates. This is consistent with a good sensitivity and reliability of biochemical bone markers.

In the patients monitored for one year, laboratory markers, and particularly DPD, reflected the status of bone disease well. In fact serial determinations showed that patients with a stable disease had essentially unchanged values, those treated with bisphosphonates showed a reduction of DPD and BGP levels, while those in whom osteolysis occurred showed a striking increment of both parameters.

In conclusion, BGP and DPD are useful in evaluating bone metabolism in MM patients. DPD can also be used to monitor skeletal involvement. These biochemical parameters represent a simple tool to identify: 1) from among patients without radiologic evidence of lytic lesions, those who deserve more accurate radiologic examinations (namely MRI); 2) from among asymptomatic patients, and in association with spinal MRI, those patients at higher risk of progression who might benefit from early treatment.

### Contributions and Acknowledgments

*AC was responsible for the conception and design of the study, data handling and writing of the manuscript. LC and SM contributed to the design, data handling and writing of the paper. CA, EO, AL, FP and CP enrolled the patients and contributed to the data collection and handling. CK performed the statistical analyses, ACS was responsible for the laboratory analysis procedures. ML contributed to the review of the paper. The order of the authors reflects the time, work and scientific contribution of all the authors.*

### Disclosures

*Conflict of interest: none.*

*Redundant publications: no substantial overlapping with previous papers.*

### Manuscript processing

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### Potential implications for clinical practice

We tried to define the role of biochemical markers, their utility in defining and monitoring bone disease in myeloma patients and their possible new role in association with MRI study in identifying, from within the group of asymptomatic patients, those who would probably benefit from early treatment.

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