Serum Levels of Carboxy-terminal Telopeptide of Type-I Collagen Are Elevated in Patients with Multiple Myeloma Showing Skeletal Manifestations in Magnetic Resonance Imaging but Lacking Lytic Bone Lesions in Conventional Radiography

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ABSTRACT

Purpose: **Skeletal involvement is a hallmark of multiple myeloma. Increased bone resorption can even be present in patients lacking osteolyses in conventional radiography. Magnetic resonance imaging (MRI) of the spine was established as a more sensitive technique to depict bone abnormalities. Type-I collagen degradation product carboxy-terminal telopeptide of type-I collagen (ICTP) was introduced as a novel biochemical parameter reflecting the bone resorption activity in myeloma. The aim of this study was to evaluate whether increased ICTP serum levels predict abnormal MRI patterns in myeloma patients.**

Experimental design: **MRI of the spine was performed in 32 untreated patients with multiple myeloma, who had no skeletal abnormalities in conventional radiographies. Simultaneously, ICTP was measured in serum by a competitive radioimmunoassay at corresponding time points.**

Results: **Serum ICTP was significantly ($P = 0.002$) elevated in patients with abnormal bone MRI compared with those patients with normal MRI findings. The sensitivity of ICTP for depiction of MRI abnormalities was 79%; the positive and negative predictive values were 85 and 84%, respectively. Compared with ICTP, the parameters of disease activity, β2-microglobulin and C-reactive protein, had a much lower sensitivity for abnormal MRI (29 and 64%, respectively).**

Conclusions: **In myeloma patients without osteolytic lesions in conventional radiography, abnormal skeletal MRI is accompanied by an increase in serum levels of ICTP. Our data show that ICTP can be used as an inexpensive parameter to identify myeloma patients with normal skeletal survey who have a high probability of skeletal involvement and deserve more accurate diagnostic evaluation using MRI.**

INTRODUCTION

Although dose-intensive chemotherapy regimes have been established during the last 20 years (1), multiple myeloma remains an incurable disease in most cases. For myeloma patients, the osteolytic bone destruction is a major clinical problem, which negatively affects their quality of life. About 75% of the myeloma patients have skeletal involvement with bone pain, lytic lesions, diffuse osteoporosis, or pathologic fractures at the time of diagnosis, and almost all patients develop bone manifestations in the later clinical course (2). Most common osteolytic lesions include the central skeleton, skull, and the femur, whereas in ~15% of the patients, diffuse osteopenia is the only bone manifestation. The standard diagnostic procedure for the detection of skeletal affections is conventional radiography. Because histomorphometric studies have shown that abnormal bone degradation can be existent even in the absence of osteolytic lesions in skeletal radiography (3), the diagnostic sensitivity of conventional X-ray appears to be low in early myeloma. Thus, MRI[2] was established as a noninvasive technique, which can recognize bone abnormalities in multiple myeloma patients with greater sensitivity than conventional radiography (4, 5) or bone densitometry (6). Furthermore, it was shown that abnormal MRI has a prognostic relevance in myeloma patients (7) and can predict progression-free survival in asymptomatic myeloma (6, 8, 9). In addition, MRI changes were identified to correlate well with response to chemotherapy in multiple myeloma patients (10).

In addition to imaging techniques, new biochemical parameters have been evaluated for monitoring the present bone resorption activity in multiple myeloma. The degradation product of type-I collagen, ICTP, has been reported to have diagnostic and prognostic relevance in cancer-induced bone disease and seems to be a suitable parameter, especially in detecting collagen degradation under pathological conditions (11). ICTP has a significant correlation with histomorphometric parameters of bone destruction in myeloma patients (12). Furthermore, it

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[2] The abbreviations used are: MRI, magnetic resonance imaging; ICTP, COOH-terminal telopeptide of type-I collagen; β2-MG, β2-microglobulin; CRP, C-reactive protein; ROC, receiver operating characteristic; Dpd, deoxycarboxy pyridinoline; RANK, receptor activator of nuclear factor κB.

Table 1 Patient characteristics

Patient characteristics	No. of patients
Total	32
Sex	
Male	19
Female (pre/postmenopausal)	13 (0/13)
Age	
Median	63
Range	46–87
Type of myeloma	
IgA	5
IgG	25
Bence-Jones-myeloma	2
Durie and Salmon Stage	
Stage I	25
Stage II	1
Stage III	6
MRI	
Normal	18
Focal/diffuse/focal and diffuse	3/5/6
ICTP	
≤Normal	19
>Normal	13

was demonstrated that ICTP serum levels are significantly elevated in myeloma patients compared with control individuals (13) and that serum-ICTP increases with the progression of the disease and decreases in patients responding to chemotherapy (14). In addition, ICTP was identified to be a prognostic factor for survival in myeloma patients (13, 15). A recent study demonstrated that ICTP serum values are increased parallel to clinical and radiographic signs of bone affection in conventional X-rays (16).

The sensitivity and specificity of collagen-I degradation products in identifying bone destruction in myeloma patients with normal skeletal radiographs had not been investigated thus far. Thus, in this study, we evaluated the relation between elevated serum ICTP levels and pathologic MRI findings of the spine in myeloma patients to analyze the relevance of ICTP for predicting bone abnormalities, which cannot be visualized by conventional radiography. In addition to ICTP, the sensitivity and specificity of established parameters of myeloma activity, *i.e.,* β2-MG and CRP, were evaluated for the prediction of myeloma-induced bone disease.

MATERIALS AND METHODS

Patients. Serum samples were collected from 32 untreated patients with multiple myeloma, who had no evidence of bone affection in conventional radiography. The median age of all individuals was 63 years. Patient characteristics are shown in Table 1. The diagnosis of multiple myeloma was made according to Durie and Salmon criteria, and in addition, the stage classification was done according to Durie and Salmon (17). At the time the investigations were conducted, the patients did not have previous antimyeloma treatment. All samples were taken after informed consent was given at the time of MRI investigation. Peripheral blood was processed after venipuncture by centrifugation at 1500 × *g* for 10 min, and serum was stored at −76°C until immunoassay analysis was performed. Serum CRP and β2-MG were detected with the clinical routine laboratory tests.

MRI. MRI of the thoracic and lumbar spine was performed in a 1.5 Tesla-System (Magnetom Vision, Erlangen, Germany). After a coronal localization scan, sagittal layers with a thickness of 3–4 mm were scanned with T1-weighted spin-echo sequences, T2-weighted double-spin-echo or turbo-spin-echo sequences with fat saturation, and short-time inversion recovery sequences. The images had a field of view of 450 mm. Additional transversal T1- or T2-weighted scans parallel to the intervertebral discs were performed in fields of interest if signs for compressing extraossal soft tissue tumors and affections of the spinal canal or discus were evident in individual patients.

Immunoassay. Serum ICTP was measured by a competitive radioimmunoassay (Orion Diagnostica, Espoo, Finland) using I^{125}-labeled ICTP as tracer molecule and a polyclonal rabbit antiserum as detection reagent. The upper reference limit for healthy controls was 5 μg/liter for female and 5.2 μg/liter for male individuals. The intra and interassay concentration variants tested in a series of repetitive samples were 1 and 11% respectively, which was within the range given by the manufacturer. The assay controls supplied by the manufacturers were within the range of mean ± 2 SDs.

Statistical Methods. Statistical analyses were performed using the SPSS-Software version 10.0 (SPSS, Inc.). The Mann-Whitney *U* test was applied to compare independent groups. Sensitivity and specificity of elevated ICTP for predicting abnormal MRI were calculated by ROC analysis. For all tests, *P*s < 0.05 were considered as statistically significant.

RESULTS

Serum ICTP values were detectable in all 32 multiple myeloma patients with normal radiographic skeletal survey and were elevated in 13 of them. Fourteen patients had abnormal magnetic resonance images, 3 with a focal, 5 with diffuse, and 6 with both diffuse and focal MRI patterns. Eleven of 13 (85%) patients with elevated serum ICTP levels had abnormal MRI, whereas only 3 of 19 (16%) patients with normal ICTP levels showed pathological MRI findings. Distribution of cases with ICTP, CRP, and β2-MG elevated/normal *versus* MRI abnormal/normal is shown in Table 2. The increase in ICTP levels was highly significant ($P = 0.002$) between the patients with bone involvement compared to those without bone involvement detected by MRI (Fig. 1). The sensitivity and specificity of elevated ICTP in predicting pathological MRI findings were 79 and 89%, respectively. An ROC analysis is shown in Fig. 2. The positive and negative predictive values for ICTP in predicting pathological MRI findings were 85 and 84%, respectively. Remarkably, 2 patients had elevated ICTP serum levels and normal MRI of the spine. Both patients developed osteolytic lesions during the later disease course, 1 of them in other regions than the spine (right femur and pelvis).

β2-MG levels did not significantly differ between patients with pathological MRI compared with those with normal MRI findings. Although the specificity of this parameter was with 89% as high as ICTP, the sensitivity was with 29% very low. The sensitivity of CRP as a surrogate marker for skeletal abnormalities in MRI was only 64%, a much lower value in

Table 2 ICTP, CRP, and β2-MG elevated/≤ normal *versus* MRI abnormal/normal

	ICTP ≤ normal	ICTP > normal	CRP ≤ 0.6 mg/liter	CRP > 0.6 mg/liter	β2-MG ≤ 2.5 mg/liter	β2-MG > 2.5 mg/liter
Normal MRI						
Stage I	16	2	17	1	16	2
Stages II & III	0	0	0	0	0	0
Total	16	2	17	1	16	2
Abnormal MRI						
Stage I	3	4	4	3	7	0
Stages II & III	0	7	2	5	3	4
Total	3	11	6	8	10	4

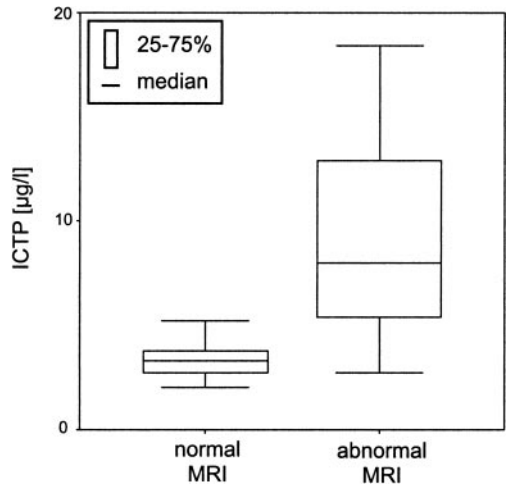

Fig. 1 Boxplots of ICTP serum levels in two groups of multiple myeloma patients: normal *versus* abnormal MRI. Mann-Whitney *U* test: $P = 0.002$.

comparison with ICTP. The specificity of CRP was 94%. The comparison of the parameters ICTP, β2-MG, and CRP is shown in Table 3. The ROC analysis revealed that ICTP had the greatest area under the curve in comparison with β2-MG and CRP and was the best parameter among those tested.

DISCUSSION

Osteolytic bone destruction is one of the major characteristics of multiple myeloma. Recent studies showed that human myeloma cells express a potent osteoclastogenic factor, the RANK ligand (18, 19). RANK ligand activates its specific receptor RANK, which is located on osteoclasts (20). The level of RANK ligand expression on the surface of bone marrow plasma cells correlated with the bone status of the patients. Myeloma patients with osteolytic bone lesions showed a significantly higher expression of RANK ligand compared with patients without osteolysis (21). The detection and monitoring of bone lesions are important diagnostic requirements for the clinical management of myeloma patients. The standard technique for the evaluation of bone involvement in multiple myeloma is skeletal radiography. Because conventional X-ray can be insufficient, especially in early myeloma, studies on different imaging modalities have demonstrated that MRI techniques reach a much higher sensitivity in depicting bone abnormalities (5, 8). Furthermore, the prognostic relevance of abnormal MRI for disease progression was demonstrated (22, 23). In accordance with these results, the clinical impact of MRI investigations in myeloma patients was verified by our data, which demonstrated that in 14 of 32 myeloma patients with normal conventional skeletal X-rays, pathological MRI patterns could be found.

Although MRI is a useful and sensitive tool to discover myeloma-induced bone disease, this technique remains expensive and is not always available. In addition to imaging techniques, biochemical parameters of collagen degradation, such as ICTP and Dpd, were established as markers reflecting bone resorption activity. Dpd was found to be significantly elevated in multiple myeloma patients (24). Dpd was recently reported to be elevated in 10 of 15 myeloma patients with MRI abnormalities, who did not show osteolytic lesions in conventional X-ray (25), but the sensitivity and specificity of elevated Dpd values were not given in this report. Recent investigations suggest that ICTP is a serum marker relatively insensitive to variations in normal bone turnover (11), whereas urinary Dpd and amino-terminal collagen type-I telopeptide appear to be more sensitive to variations of physiological bone turnover, like age and menopausal status (26, 27). A possible reason for these differences is the fact that the cleavage points of the ICTP molecule are mainly affected by proteases, which are activated under pathological conditions (11). Thus, ICTP could preferentially be used to monitor pathological type I collagen degradation in multiple myeloma. In a comparative study of serum ICTP and urinary Dpd and amino-terminal collagen type-I telopeptide levels in multiple myeloma, we found that serum ICTP was the best prognostic factor for survival among these bone resorption parameters investigated (28). A correlation of clinical and radiographic signs of bone involvement with ICTP serum levels was shown previously in myeloma patients (13, 16), but only conventional radiography was evaluated in these studies. Although the measurement of collagen degradation products may be a sensitive diagnostic tool in evaluating myeloma-induced bone disease, there are no data available on the sensitivity and specificity of these markers for predicting MRI abnormalities. Our study demonstrates for the first time that elevated serum ICTP is significantly correlated with MRI abnormalities in myeloma patients without osteolytic lesions in conventional radiography. Elevated ICTP levels can predict abnormal MRI findings with a sensitivity and specificity of 79 and 89%, respectively. The comparison of ICTP with the established parameters of disease activity shows that β2-MG and CRP are less suitable in predicting myeloma-induced MRI abnormalities. The sensitivity of

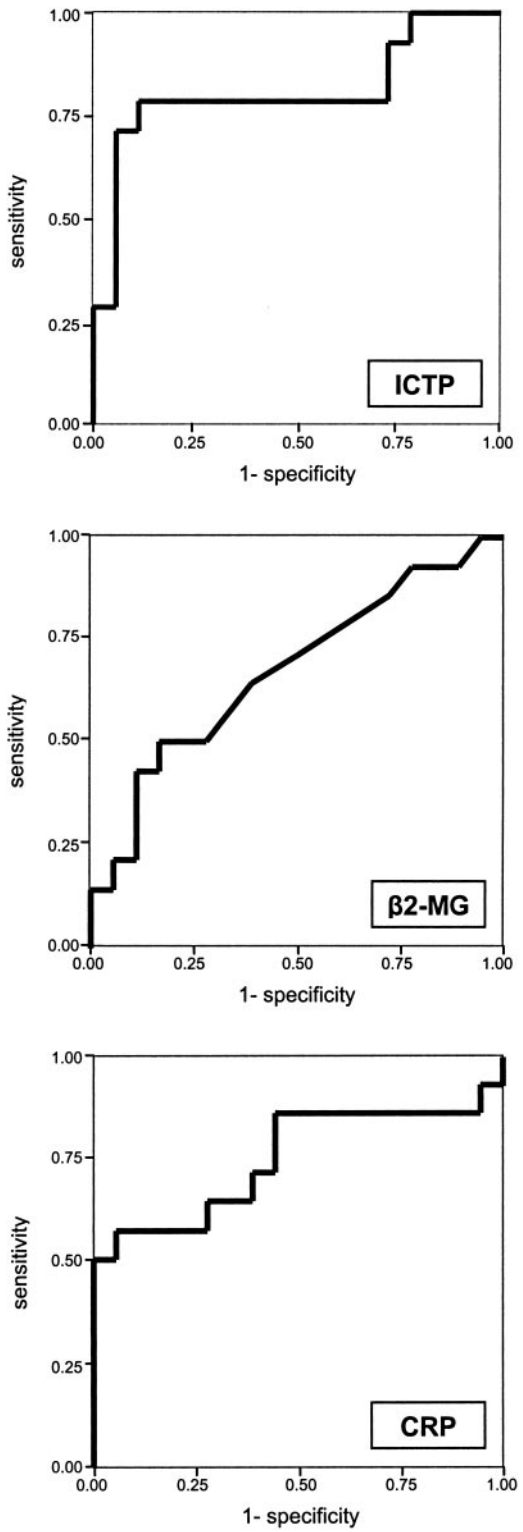

Fig. 2 ROC curve for the relation of elevated serum ICTP, β2-MG, or CRP levels and abnormal MRI findings in myeloma patients.

Table 3 Sensitivity and specificity of elevated values, area under the curve of the ROC curve, and predictive values for ICTP, CRP, and β2-MG for depiction of skeletal involvement in MRI

	ICTP	CRP	β2-MG
Sensitivity	79%	64%	29%
Specificity	89%	94%	89%
AUC[a]	0.81	0.746	0.675
Positive predictive value	85%	90%	67%
Negative predictive value	84%	77%	62%

[a] AUC, area under the curve.

both β2-MG (29%) and CRP (64%) in predicting abnormal MRI findings is low; thus, these parameters cannot be used as surrogate markers of myeloma-induced bone disease in the clinical setting.

Both ICTP and skeletal MRI were identified as sensitive diagnostic tools for the detection of myeloma-induced bone disease. Our results suggest that elevated serum ICTP can be used as a reasonable additive tool in identifying myeloma patients, who deserve a more intensive diagnostic procedure and follow-up despite negative skeletal X-rays. Considering the high negative predictive value of ICTP for abnormal MRI results, ICTP may be used to avoid expensive MRI diagnostic for patients with normal ICTP levels.

REFERENCES

1. Barlogie, B. Advances in therapy of multiple myeloma: lessons from acute leukemia. Clin. Cancer Res., *3:* 2605–2613, 1997.

2. Kyle, R. A. Multiple myeloma: review of 869 cases. Mayo Clin. Proc., *50:* 29–40, 1975.

3. Bataille, R., Chappard, D., Marcelli, C., Rossi, J. F., Dessauw, P., Baldet, P., Sany, J., and Alexandre, C. Osteoblast stimulation in multiple myeloma lacking lytic bone lesions. Br. J. Haematol., *76:* 484–487, 1990.

4. Moulopoulos, L. A., and Dimopoulos, M. A. Magnetic resonance imaging of the bone marrow in hematologic malignancies. Blood, *90:* 2127–2147, 1997.

5. Dimopoulos, M. A., Moulopoulos, L. A., Datseris, I., Weber, D., Delasalle, K., Gika, D., and Alexanian, R. Imaging of myeloma bone disease–implications for staging, prognosis and follow-up. Acta Oncol., *39:* 823–827, 2000.

6. Mariette, X., Zagdanski, A. M., Guermazi, A., Bergot, C., Arnould, A., Frija, J., Brouet, J. C., and Fermand, J. P. Prognostic value of vertebral lesions detected by magnetic resonance imaging in patients with stage I multiple myeloma. Br. J. Haematol., *104:* 723–729, 1999.

7. Kusumoto, S., Jinnai, I., Itoh, K., Kawai, N., Sakata, T., Matsuda, A., Tominaga, K., Murohashi, I., Bessho, M., Harashima, K., and Heshiki, A. Magnetic resonance imaging patterns in patients with multiple myeloma. Br. J. Haematol., *99:* 649–655, 1997.

8. Moulopoulos, L. A., Dimopoulos, M. A., Smith, T. L., Weber, D. M., Delasalle, K. B., Libshitz, H. I., and Alexanian, R. Prognostic significance of magnetic resonance imaging in patients with asymptomatic multiple myeloma. J. Clin. Oncol., *13:* 251–256, 1995.

9. Weber, D. M., Dimopoulos, M. A., Moulopoulos, L. A., Delasalle, K. B., Smith, T., and Alexanian, R. Prognostic features of asymptomatic multiple myeloma. Br. J. Haematol., *97:* 810–814, 1997.

10. Moulopoulos, L. A., Dimopoulos, M. A., Alexanian, R., Leeds, N. E., and Libshitz, H. I. Multiple myeloma: MR patterns of response to treatment. Radiology, *193:* 441–446, 1994.

11. Sassi, M. L., Eriksen, H., Risteli, L., Niemi, S., Mansell, J., Gowen, M., and Risteli, J. Immunochemical characterization of assay for car-

boxyterminal telopeptide of human type I collagen: loss of antigenicity by treatment with cathepsin K. Bone, *26:* 367–373, 2000.

12. Abildgaard, N., Glerup, H., Rungby, J., Bendix-Hansen, K., Kassem, M., Brixen, K., Heickendorff, L., Nielsen, J. L., and Eriksen, E. F. Biochemical markers of bone metabolism reflect osteoclastic and osteoblastic activity in multiple myeloma. Eur. J. Haematol., *64:* 121–129, 2000.

13. Carlson, K., Larsson, A., Simonsson, B., Turesson, I., Westin, J., and Ljunghall, S. Evaluation of bone disease in multiple myeloma: a comparison between the resorption markers urinary deoxypyridinoline/creatinine (DPD) and serum ICTP, and an evaluation of the DPD/osteocalcin and ICTP/osteocalcin ratios. Eur. J. Haematol., *62:* 300–306, 1999.

14. Elomaa, I., Virkkunen, P., Risteli, L., and Risteli, J. Serum concentration of the cross-linked carboxyterminal telopeptide of type I collagen (ICTP) is a useful prognostic indicator in multiple myeloma. Br. J. Cancer, *66:* 337–341, 1992.

15. Abildgaard, N., Bentzen, S. M., Nielsen, J. L., and Heickendorff, L. Serum markers of bone metabolism in multiple myeloma: prognostic value of the carboxy-terminal telopeptide of type I collagen (ICTP). Nordic Myeloma Study Group (NMSG). Br. J. Haematol., *96:* 103–110, 1997.

16. Fonseca, R., Trendle, M. C., Leong, T., Kyle, R. A., Oken, M. M., Kay, N. E., Van Ness, B., and Greipp, P. R. Prognostic value of serum markers of bone metabolism in untreated multiple myeloma patients. Br. J. Haematol., *109:* 24–29, 2000.

17. Durie, B. G., and Salmon, S. E. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival. Cancer (Phila.), *36:* 842–854, 1975.

18. Sezer, O., Heider, U., Jakob, C., Eucker, J., and Possinger, K. Human bone marrow myeloma cells express RANKL. J. Clin. Oncol., *20:* 353–354, 2002.

19. Sezer, O., Heider, U., Jakob, C., Zavrski, I., Eucker, J., Possinger, K., Sers, C., and Krenn, V. Immunocytochemistry reveals RANKL expression of myeloma cells. Blood, *99:* 4646–4647, 2002.

20. Sezer, O., Heider, U., Zavrski, I., Kühne, C. A., and Hofbauer, L. C. RANK ligand and osteoprotegerin in myeloma bone disease. Blood, *101:* 2094–2098, 2003.

21. Heider, U., Langelotz, C., Jakob, C., Zavrski, I., Fleissner, C., Eucker, J., Possinger, K., Hofbauer, L. C., and Sezer, O. Expression of receptor activator of NF-κB ligand (RANKL) on bone marrow plasma cells correlates with osteolytic bone disease in patients with multiple myeloma. Clin. Cancer Res., *9:* 1436–1440, 2003.

22. Facon, T., Menard, J. F., Michaux, J. L., Euller-Ziegler, L., Bernard, J. F., Grosbois, B., Daragon, A., Azais, I., Courouble, Y., Kaplan, G., *et al.* Prognostic factors in low tumour mass asymptomatic multiple myeloma: a report on 91 patients. The Groupe d'Etudes et de Recherche sur le Myelome (GERM). Am. J. Hematol., *48:* 71–75, 1995.

23. Vande Berg, B. C., Michaux, L., Lecouvet, F. E., Labaisse, M., Malghem, J., Jamart, J., Maldague, B. E., Ferrant, A., and Michaux, J. L. Nonmyelomatous monoclonal gammopathy: correlation of bone marrow MR images with laboratory findings and spontaneous clinical outcome. Radiology, *202:* 247–251, 1997.

24. Pecherstorfer, M., Seibel, M. J., Woitge, H. W., Horn, E., Schuster, J., Neuda, J., Sagaster, P., Kohn, H., Bayer, P., Thiebaud, D., and Ludwig, H. Bone resorption in multiple myeloma and in monoclonal gammopathy of undetermined significance: quantification by urinary pyridinium cross-links of collagen. Blood, *90:* 3743–3750, 1997.

25. Corso, A., Arcaini, L., Mangiacavalli, S., Astori, C., Orlandi, E., Lorenzi, A., Passamonti, F., Klersy, C., Pascutto, C., Canevari-Sciorati, A., and Lazzarino, M. Biochemical markers of bone disease in asymptomatic early stage multiple myeloma. A study on their role in identifying high risk patients. Haematologica, *86:* 394–398, 2001.

26. Sone, T., Miyake, M., Takeda, N., and Fukunaga, M. Urinary excretion of type I collagen crosslinked N-telopeptides in healthy Japanese adults: age- and sex-related changes and reference limits. Bone, *17:* 335–339, 1995.

27. Del Campo, M. T., Gonzalez-Casaus, M. L., Aguado, P., Bernad, M., Carrera, F., and Martinez, M. E. Effects of age, menopause and osteoporosis on free, peptide-bound and total pyridinium crosslink excretion. Osteoporos. Int., *9:* 449–454, 1999.

28. Jakob, C., Zavrski, I., Heider, U., Brux, B., Eucker, J., Langelotz, C., Sinha, P., Possinger, K., and Sezer, O. Bone resorption parameters carboxy-terminal telopeptide of type-I collagen (ICTP), amino-terminal collagen type-I telopeptide (NTx) and deoxypyridinoline (Dpd) in MGUS and multiple myeloma. Eur. J. Haematol., *69:* 37–42, 2002.

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