

Elevated Serum Concentration of Hepatocyte Growth Factor in Patients With Multiple Myeloma: Correlation With Markers of Disease Activity

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Hepatocyte growth factor (HGF) has been shown to be involved in angiogenesis, epithelial cell proliferation, and osteoclast activation. HGF and its receptor are expressed on myeloma cell lines and could be involved in the pathogenesis of bone destruction in multiple myeloma (MM). The aim of this study was to examine serum levels of HGF in untreated MM patients and its correlation with bone turnover indices and markers of disease activity. Forty-seven newly diagnosed MM patients and 25 controls were included: 12 patients were of stage I, 13 of stage II, and 22 of stage III (Durie–Salmon classification). Bone lesions were scored from 0 to 3, according to X-ray findings. Serum osteocalcin (OC), interleukin-6 (IL-6), TNF- α , β_2 -microglobulin (β_2 M), CRP, calcium, and 24-hr urine N-telopeptide cross-links of collagen breakdown (NTx) were determined. HGF levels were significantly higher at stage III compared to stages II and I (medians: 1,990.4 vs. 1,743.8 and 1,432.4 pg/mL, respectively, $P < 0.05$). Similarly, NTx, IL-6, TNF- α , CRP, β_2 M, and calcium increased significantly with advancing stage ($P < 0.01$). OC was higher at stage I in comparison to stages II and III ($P < 0.01$). All parameters were significantly higher in patients than controls. HGF showed a strong correlation with IL-6 and TNF- α and less with β_2 M, CRP, NTx, and OC. We conclude that serum HGF levels are increased in advanced stages of MM disease and extended bone lesions. HGF correlates with IL-6 and TNF- α , which are cytokines involved in osteoclast stimulation in MM. However, an independent association of HGF with bone turnover markers was not shown in this study, thus its role in MM bone disease needs to be further clarified. *Am. J. Hematol.* 72:229–233, 2003. © 2003 Wiley-Liss, Inc.

Key words: hepatocyte growth factor; osteocalcin; N-telopeptide; multiple myeloma

INTRODUCTION

Multiple myeloma is a plasma cell disorder characterized by the clonal expansion of malignant plasma cells in the bone marrow. It is associated with several clinical manifestations, such as osteolysis, anemia, hypercalcemia, and renal dysfunction. The production of various cytokines by myeloma cells is implicated in the production of these features.

Hepatocyte growth factor (HGF) was originally identified as a distinct cytokine that promotes hepatocyte cell growth [1]. It is also known as a mitogenic factor involved in blood vessel formation [2] and as a promoter of cell proliferation and invasion by causing destruction of

tight junctions. Because of these properties HGF is also called a scatter factor [3].

A trans membrane tyrosine kinase that is encoded by the proto-oncogene *c-met* is the HGF receptor [4] and is expressed in myeloma cell lines, on freshly isolated my-

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eloma cells [5] as well as on other cells of the bone marrow [6]. In addition to its ability to promote angiogenesis, HGF stimulates the formation of osteoclasts from hematopoietic precursor cells and attracts osteoclasts to the site of bone resorption, which, in coculture with osteoblasts, increases the rate of bone resorption [7,8]. In a previous report, it was shown that the HGF receptor is expressed by human primary osteoclasts, by osteoclast-like cell lines and by osteoblasts [8].

Interestingly, osteoclasts were found to synthesize and secrete biologically active HGF, suggesting an autocrine regulation of osteoclasts by HGF and a paracrine regulation of osteoblasts from the HGF produced by osteoclasts [8]. This was supported by another study where it was shown that, on resorptive sites, the response of osteoclasts to HGF is modulated by the presence of osteoblastic cells [9].

Despite the knowledge obtained from cell cultures regarding HGF, little is known about its role in MM *in vivo*. The purpose of this study was to evaluate serum levels of HGF in patients with MM with varying severity of disease. Furthermore, we examined the relation between HGF at diagnosis and some prognostic biological parameters and markers of bone resorption.

MATERIALS AND METHODS

Patients

We studied 47 previously untreated multiple myeloma patients, 12 in stage I, 13 in stage II and 22 in stage III according to the Durie–Salmon classification [10]. Twenty-five age- and sex-matched healthy volunteers from the staff of the hospital were used as controls. Informed consent was obtained from all subjects.

The median age of the patients in the study group was 64 years (range 38–83 years). There were 26 males (age range 36–83 years) and 21 females (age range 34–81 years). The paraprotein heavy chain was IgG in 27 patients and IgA in 15 patients, and 5 patients had light-chain disease. No patient had received chemotherapy or radiotherapy prior to initial sampling. None of the subjects studied had evidence of thyroid or parathyroid hormone-related abnormalities or had received any therapy known to affect bone metabolism. Bone involvement was graded, according to standard X-ray evaluation, into three scores: no lesions (score 0), one bone involved or diffuse osteoporosis (score 1), more than one but less than four bone lesions (score 2), and more than four bone lesions or bone fracture present (score 3). Using these definitions, 11 patients had score 0, 10 had score 1, 10 had score 2, and 16 presented with extensive disease (score 3).

Serum samples were collected at diagnosis, under sterile conditions. Urine samples were collected over 24 hr and maintained at refrigerator temperature during the

collection. All specimens were frozen at -70°C after collection and assayed at the end of the study in order to avoid inter-assay variability. Urine creatinine was determined to ensure that a 24-hr urine specimen had been collected and to normalize the results.

Biochemical Markers Assay

Serum osteocalcin (OC) values were determined by immunoassay (Novo calcin, METRA Biosystems, Mountain View, CA).

Urine N-telopeptide (NTx) cross-links of type I collagen was measured by a competitive inhibition enzyme-linked immunosorbent assay (Osteomark, Ostex International, Seattle, WA), using a monoclonal antibody for NTx labeled with horseradish peroxidase.

Serum β_2 -microglobulin ($\beta_2\text{M}$) was measured with a micro-ELISA method using the commercially available kit from Abbott (IMX, Abbott Park, IL). Serum CRP levels were measured by nephelometry (Dade Behring GmbH, Marburg, Germany). Serum calcium was measured using the respective method routinely applied in the clinical chemistry laboratory of the hospital.

Measurements of HGF, IL-6, and TNF- α in serum samples collected from patients and controls were performed by solid-phase sandwich enzyme-linked immunosorbent assay (ELISAS) with commercially available test kits (Quantikine Human HGF, IL-6, and TNF- α , R&D Systems, Minneapolis, MN).

Bone marrow cellularity and percentage of bone marrow infiltration by myeloma cells was estimated by bone marrow biopsies from the posterior iliac crests.

Statistical Analysis

All parameters are expressed as medians and ranges (min–max). Statistical analyses for stages I–III and for bone lesion scores 0–3 were performed using the non-parametric Kruskal–Wallis test to evaluate differences among the parameters studied. Statistical comparison between the MM group and the control group was made using the non-parametric Mann–Whitney test. The correlation between HGF and the studied parameters was evaluated by Spearman rank correlation coefficient. A *P* value of less than 0.05 was considered significant, as adjusted by Bonferroni correction for multiple comparisons.

RESULTS

Serum Concentrations of HGF and Markers of Bone Disease

In the group of MM patients serum HGF values ranged from 724.4 to 16,671.4 pg/mL with a median of 1,623.3 pg/mL. The median HGF in the control group was 468.2 pg/mL.

Serum HGF concentrations of patients with MM were

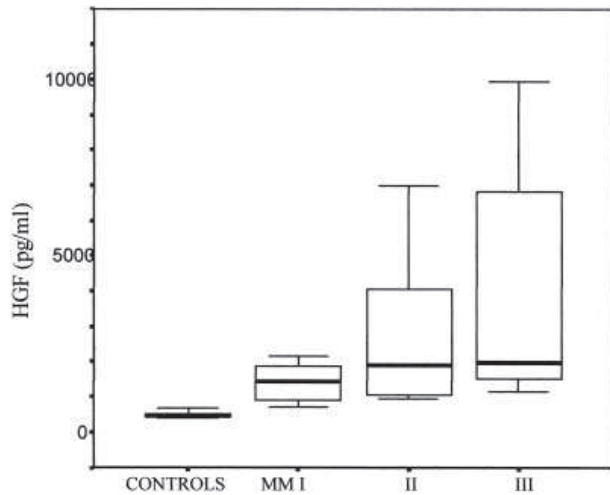


Fig. 1. Levels of HGF according to stage in MM patients and controls: the thick bars represent the median values; boxes represent 25–75% of values; and the range bars represent the 95% confidence intervals.

significantly elevated in comparison to controls ($P < 0.01$). Furthermore, a statistically significant increase in HGF levels according to Durie–Salmon stage was observed (Fig. 1).

Similarly, OC, calcium serum levels, and urinary levels of NTx were significantly increased in untreated MM patients in comparison to controls ($P < 0.01$). Moreover, statistically significant differences were found among the levels of calcium, OC, and NTx in stages I and III of the MM group. Results from the analysis of the MM patients data and controls are shown in Table I.

Serum Concentrations of IL-6 and TNF- α

Serum IL-6 concentrations in MM patients differed at various stages of disease (Table I). IL-6 values ranged from 0.62 to 26.08 pg/mL (median 4.4 pg/mL). This median was significantly higher than the respective median (0.67) of controls ($P < 0.01$).

Similarly, significant differences were noted for the levels of TNF- α between patients of stage I versus stage III ($P < 0.01$). Additionally, TNF- α serum levels of MM patients were significantly increased in comparison to controls ($P < 0.01$).

Relationship Between Cytokines, Bone Markers, and Skeletal Involvement Noted on X Ray

There was a positive association between HGF, IL-6, TNF- α , and bone involvement as their levels increased with increasing bone lesion score (Table II and Fig. 2).

An association between OC serum levels and bone involvement score was also observed. OC serum levels among myeloma patients decreased with more extensive bone disease. Furthermore, NTx excretion differed sig-

nificantly within the MM patients with higher levels in patients with extensive bone involvement (score 3 versus 0 and 1, $P < 0.01$).

Correlations Between Parameters

Serum HGF correlated significantly with serum IL-6 ($r = 0.492$, $P < 0.0001$) and TNF- α ($r = 0.497$, $P < 0.0001$).

HGF correlated positively with NTx ($r = 0.426$, $P < 0.003$), and negatively with OC ($r = -0.360$, $P < 0.01$). Correlations were also observed between HGF and the percentage of plasma cell infiltration of the bone marrow, β_2 M, CRP, and serum calcium (Table III).

A positive correlation was also found between serum IL-6 and β_2 M ($r = 0.664$, $P < 0.0001$), CRP ($r = 0.288$, $P < 0.005$), TNF- α ($r = 0.617$, $P < 0.0001$), and calcium ($r = 0.389$, $P < 0.007$).

Similarly, serum TNF- α correlated significantly with β_2 M ($r = 0.570$, $P < 0.0001$) and calcium ($r = 0.431$, $P < 0.002$).

A negative correlation was found between serum OC and CRP ($r = -0.285$, $P < 0.05$), β_2 M ($r = -0.608$, $P < 0.0001$), serum TNF- α ($r = -0.355$, $P < 0.01$), NTx ($r = -0.689$, $P < 0.0001$), and IL-6 ($r = -0.438$, $P < 0.002$). No correlation was found between OC and serum calcium.

NTx correlated significantly with β_2 M ($r = 0.540$, $P < 0.0001$), IL-6 ($r = 0.457$, $P < 0.001$), and serum calcium ($r = 0.443$, $P < 0.002$). No correlation was observed between NTx and serum CRP.

DISCUSSION

This study was performed to examine the relation of HGF with MM stage, IL-6, TNF- α , and biochemical markers of bone metabolism.

Previous studies have implicated HGF in the biological responses of osteoclasts and osteoblasts [7–9]. HGF is released from inflammatory cells [11] and is produced by fibroblasts in response to inflammatory cytokines such as IL-6 [12] and TNF- α [13]. Myeloma cells can produce HGF and express the receptor for HGF [5]. It was previously shown that serum levels of HGF fluctuated in concordance to the tumor burden and supported the hypothesis of HGF as a bone-destructive cytokine in MM [14]. This study, as well as another in MM patients, [20] did not show a correlation of HGF with MM stage. The present study is the first to show that serum HGF concentrations correlate with myeloma severity as defined by Salmon and Durie staging system. Patients with stage III exhibited higher serum HGF than patients with stage I of the disease.

Furthermore, in our study, HGF correlated strongly with IL-6 and TNF- α , which are produced locally in the bone marrow microenvironment and are involved in os-

TABLE I. Median Values and Range of Measured Parameters According to Disease Stage in Patients With Multiple Myeloma and in Controls

	Stage			Controls (n = 25)
	I (n = 12)	II (n = 16)	III (n = 23)	
Infiltration (%)	15 (5–30)	40 (10–80)	55* (5–90)	—
HGF (pg/mL)	1432.4 (724.4–2161.7)	1743.8 (950.0–12347.8)	1990.4** (1128.0–16671.4)	468.2 [†] (389.7–880.9)
β_2 M (mg/L)	1.6 (0.8–1.9)	2.3 (1.3–5.8)	4.0** (2.1–10.8)	1.2 [†] (0.7–2.7)
CRP (mg/dL)	0.55 (0.50–1.55)	0.70 (0.50–2.81)	1.14*** (0.50–2.90)	0.30 [†] (0.30–0.60)
IL-6 (pg/mL)	1.72 (0.62–5.08)	2.83 (0.81–5.99)	6.56** (1.94–26.70)	0.67 [†] (0.38–2.17)
TNF- α (pg/mL)	1.54 (0.90–3.21)	2.66 (1.02–9.70)	3.67* (1.52–9.47)	1.28 [†] (0.78–2.56)
Calcium (mg/dL)	9.0 (8.4–10.5)	9.2 (8.6–10.9)	9.9** (8.1–15.0)	8.6 (7.8–9.8)
NTx (nmol BCE/mM creat)	35.5 (24.0–87.0)	170.0 (84.0–274.0)	216.0* (99.0–310.0)	31.5 [†] (21.0–39.0)
OC (ng/mL)	29 (16–39)	18 (8–24)	10* (4–30)	7.5 [†] (4–9)

*Stage III versus I, $P < 0.01$; III versus II, n.s.; II versus I, n.s.

**Stage III versus I, $P < 0.05$; III versus II, n.s.; II versus I, n.s.

***Stage III versus I, $P < 0.03$; III versus II, n.s.; II versus I, n.s.

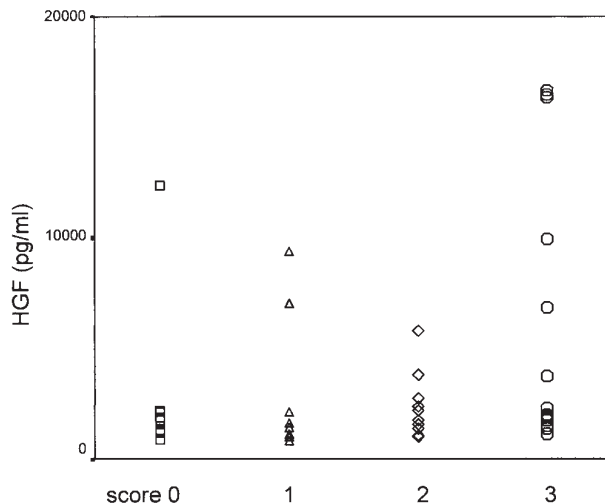
[†]Control group versus MM, $P < 0.01$.

TABLE II. Median Values and Range of Measured Cytokines According to Bone Lesion Score

	Score			
	0 (n = 11)	1 (n = 10)	2 (n = 10)	3 (n = 16)
HGF (pg/mL)	1712.8 (894.6–12347.8)	1454.9 (724.4–9333.3)	1952.8 (1017.6–5785.2)	1902.1* (199.4–16671.4)
IL-6 (pg/mL)	2.00 (0.89–5.08)	2.52 (0.62–26.70)	4.83 (1.12–24.01)	5.10 (0.81–20.50)
TNF- α (pg/mL)	2.26 (0.90–4.38)	2.38 (1.02–8.01)	3.23 (2.14–9.70)	2.87 (1.50–9.47)
OC (ng/mL)	29.5 (18–39)	19.5 (7–29)	13.5 (6–23)	10** (4–15)
NTx (nmol BCE/mM creat)	76.5 (31–99)	124.5 (27–262)	172 (142–274)	223** (197–310)

*Grouped grades 0–1 versus grade 3, $P < 0.05$; Grades 0–1 versus 2 and 2 versus 3, P non-significant (n.s.).

**Grade 0 versus grade 1, 2 and grade 0 versus 3, $P < 0.01$; grade 1 versus 2 and 3, P n.s.

**Fig. 2. HGF values according to bone destruction score by X-ray evaluation.**

teoclastic stimulation and bone resorption [15,16]. TNF- α is also a potent inducer of IL-6 production by myeloma cells [17]. IL-6 and β_2 M have been associated with survival in MM [18,19]. Our results showed a

TABLE III. Correlations (Using Spearman Correlation Coefficient) Between HGF Values and Other Parameters at MM Diagnosis

	β_2 M	IL-6	Calcium	TNF- α	NTx	OC	Infiltr.
r value	0.458	0.492	0.323	0.497	0.426	-0.360	0.411
P value	0.001	0.0001	0.02	0.0001	0.003	0.01	0.006

correlation between HGF and β_2 M, which is in accordance with previous findings [20].

The severity of the bone involvement seems to be correlated to the degree of plasma cell infiltration [21] with the production of osteoblastic activating factors from plasma cells probably in collaboration with stromal cells [22]. In this study the degree of bone destruction was evaluated by the conventional radiological method. Patients with diffuse osteoporosis (bone score 1) may have diffuse marrow infiltration without significant bone destruction. However, only 1 patient with a score of 1 had over 50% infiltration, therefore the X-ray method used in our patients evaluated satisfactorily the extent of bone disease.

Various biochemical parameters of bone metabolism have been used to provide more precise information on the actual rates of bone resorption and bone formation. A

fragment from the amino-terminal cross-linked telopeptide of type I collagen (NTx), which is excreted into the urine, has been proposed as the most specific biochemical marker of bone resorption [23,24]. On the other hand, serum osteocalcin, which is excreted by osteoblasts, reflects bone formation and has been proposed as a prognostic marker of MM disease [25,26]. We observed elevated levels of NTx and OC in MM patients with no visible bone disease on X-ray (score 0) and increased levels of NTx but decreased levels of OC in patients with advancing bone disease.

In a previous study HGF was found to be correlated to two other markers of bone resorption: C-terminal telopeptide of type I collagen (ICTP) and C-terminal propeptide of procollagen type I (PICP) [14]. In our study, we found a positive correlation between HGF and urinary levels of NTx. On the contrary, serum OC levels correlated inversely with HGF. However, these findings cannot support an independent relation of HGF with bone turnover indices, as all the parameters studied in our study were inter-correlated. From the *r* value a strong correlation was seen between HGF and the cytokines IL-6 and TNF- α . The correlation between HGF and NTx and OC may be indirect due to the strong association of HGF with IL-6, TNF- α , and the stage of disease. Multiple regression analysis of the studied parameters was not feasible due to small sample size.

In conclusion, we consider that increased serum HGF levels in patients with MM at diagnosis are related to disease stage and cytokines involved in disease progression, such as IL-6 and TNF- α . However, its involvement in bone destruction in MM needs to be clarified with further studies.

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