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Correlation of osteoprotegerin and sRANKL concentrations in serum and bone marrow of multiple myeloma patients

Maria Kraj, Katarzyna Owczarska, Urszula Sokołowska, Piotr Centkowski, Ryszard Pogłód and Barbara Kruk

Department of Hematology, Institute of Hematology and Blood Transfusion, Warsaw, Poland

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Summary

Introduction: Recent studies suggest that multiple myeloma (MM) triggers osteoclastogenesis by disrupting the balance between the receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG), its natural antagonist.

Materials and Methods: Determinations of bone marrow (BM) and serum OPG and sRANKL concentrations were performed in 133 MM patients and 42 healthy subjects by the ELISA method using Osteoprotegerin ELISA and sRANKL ELISA kits.

Results: MM patients had elevated serum levels of OPG compared with controls ($p<0.0001$) and OPG levels were higher in patients with renal failure and patients with hipercalcemia ($p<0.001$ and $p=0.04$, respectively). Serum OPG levels correlated with age, serum β_2-microglobulin, and BM OPG concentrations and did not correlate with the presence of osteolysis or with stage of disease. sRANKL serum levels in MM patients and in controls were not statistically different ($p=0.42$). In MM patients, serum OPG and sRANKL levels were similar at diagnosis and in the plateau phase of disease. There was a correlation between BM and serum sRANKL concentrations ($p<0.001$). Median values of the sRANKL/OPG ratio for BM and serum of MM patients were 0.14 and 0.11, respectively. The median value of the sRANKL/OPG ratio for the serum of controls was 0.11.

Conclusion: In 20% of MM patients, serum OPG levels are elevated, and this may be a compensative reaction related to increased bone destruction. There is not statistically significant relationship between sRANKL serum and BM levels and the main clinical and laboratory parameters of the disease. Determination of BM and the serum sRANKL/OPG ratio seems to have no clinical value.

Key words: **multiple myeloma • osteoprotegerin • RANKL • osteolysis**

Abbreviations: **MM** – multiple myeloma, **OPG** – osteoprotegerin, **sRANKL** – receptor activator of NK-κB ligand, **BM** – bone marrow.

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Author's address: Prof. Maria Kraj, M.D. Ph.D., Department of Hematology, Institute of Hematology and Blood Transfusion, Chocimska 5, 00-957 Warsaw, Poland, tel./fax: +48 22 84-98-432, e-mail: mkraj@ihit.waw.pl

INTRODUCTION

Multiple myeloma (MM) is characterized by the accumulation of malignant plasma cells in the bone marrow (BM) and the development of skeletal disease as a result of an increased activity of osteoclasts that leads to increased bone resorption in combination with decreased bone formation. The interaction of myeloma plasma cells with BM stromal cells in the BM microenvironment is crucial for the activation of osteoclasts[53]. A number of osteoclast-activating factors produced by stromal and myeloma cells have been identified, including tumor necrosis factor (TNF)-α and -β, interleukin (IL)-1β, IL-6, vascular endothelial growth factor, and macrophage inflammatory protein-1α and -β[25]. An essential cytokine system for osteoclast differentiation and activation consists of the receptor activator of NK-κB ligand (RANKL)[1, 23, 31, 52, 55], its receptor RANK[1], and the soluble decoy receptor osteoprotegerin (OPG)[46]. RANKL is produced mainly by osteoblasts and marrow stromal cells as a membrane-bound protein; subsequently, it is cleaved into a soluble form (sRANKL) by the metalloprotease-disintegrin TNF--α-converting enzyme[24]. In addition, activated T cells secrete RANKL as a soluble molecule[17, 24]. The interaction of RANKL with RANK, which is expressed on the surface of osteoclast precursor cells and osteoclasts[7], stimulates the differentiation and activation of osteoclasts, whereas OPG blocks this process by functioning as a decoy receptor for RANKL[35, 46]. The balance between the expressions of RANKL and OPG, a naturally occurring soluble glycoprotein produced by many different cell types including marrow stromal cells and osteoblasts, determines the level of osteoclast formation and the extent of bone resorption.

RANKL-mediated mechanisms of activation of osteoclastogenesis have been described for a variety of malignant tumors capable of forming skeletal metastases or causing hypercalcemia, including breast cancer, prostate cancer[4, 5], squamous cell carcinoma[38], adult T cell leukemia[39], Hodgkin's disease[15], and neuroblastoma[34]. Systemic RANKL blockage using OPG, OPG-Fc fusion protein, or inhibitory RANK antibodies has been successfully used to treat osteolytic metastases[21, 34, 37, 56], humoral hypercalcemia[8, 40], and tumor-associated bone pain[21, 33] in various animal models of non-myeloma malignancies.

Recent studies suggest that in MM patients there is a marked imbalance between RANKL expression and OPG levels that favors osteoclastogenesis and osteoclast activation. Several studies have demonstrated that myeloma cells enhance RANKL expression by bone marrow stromal cells through direct cell-to-cell contact[16, 41, 43], up-regulate RANKL production by activated T cells[17], and inhibit OPG production[16, 41]. Several studies reported RANKL expression by myeloma cells using either human primary myeloma cells from patients[18, 45] or the murine myeloma cell line 5T2MM[12]. The expression of RANKL on BM plasma cells correlates with osteolytic bone disease in MM patients[14, 18]. Terpos et al.[50] have shown that the RANKL/OPG ratio is increased in MM patients, correlates with the extent of bone disease, and has an impact on survival. In experimental studies, the blockage of RANKL by RANK-Fc antibody, a recombinant RANKL antagonist, in myeloma mice models inhibited not only bone resorption, but also myeloma cell growth[41, 47, 53]. Moreover, recombinant OPG administration in murine models of MM resulted in decreased tumor growth and increased survival[51]. In humans, recombinant OPG has caused suppression of bone resorption in menopausal women with osteoporosis[2] and in patients with MM and breast cancer[3].

The aim of the present study was to evaluate the serum and BM OPG and sRANKL concentrations in patients with MM and assess their clinical and laboratory correlations. Preliminary results of our research have already been presented at the 9th Congress of the European Hematology Association, 20th Congress of the Polish Society of Hematology and Blood Transfusion[26], 12th International Congress of Immunology and 4th Annual Conference of FOCIS[27], and at the 10th International Myeloma Workshop[28].

MATERIALS AND METHODS

The study included 133 newly diagnosed and untreated MM patients (61 women and 72 men with ages ranging 31–86 years; 24 at disease stage I, 20 at II, and 89 at III according to D.S.; 92 having lytic lesions at skeletal X-ray survey, 11 having hypercalcemia, and 18 renal failure; monoclonal protein IgG was found in 82 patients, IgA in 32, IgD in 2, IgM in 1, Bence Jones in 13, and non-secretory in 3), 10 MM patients in the plateau phase of disease, 4 plasma cell leukemia patients, and 3 patients with monoclonal gammopathy of undetermined significance (MGUS) hospitalized in the Department of Hematology of the Institute of Hematology and Blood Transfusion in Warsaw.

In each patient the following parameters were estimated at the time of diagnosis: age, sex, complete blood examination with differential, percentage of

plasma cells in the BM, monoclonal protein isotype (using the Beckman Paragon Immunofixation Electrophoresis Kit, USA), urine immunoglobulin/24 h, serum concentrations of monoclonal protein, calcium, creatinine, and β_2-microglobulin (using the Beckman ARRAY 360, USA), complete X-ray skeletal survey, and disease staging according to the Durie and Salmon classification. The patients' informed consent was obtained prior to performing BM aspiration or biopsy.

The control group was 42 healthy subjects (recruited among blood donors, our institute staff, and their family members): 22 women and 20 men, at ages ranging 22–81 years. None of he controls were receiving medications or had medical conditions known to affect bone metabolism. The study was conducted with Ethical Committee approval.

Serum and BM OPG and sRANKL were measured by enzyme-linked immunosorbent assay (ELISA) using kits manufactured by Biomedica GmbH (Vienna, Austria). The tests were performed according to the manufacturer's instructions and previously published results[10]. The tests were performed in serum and BM samples which were undiluted and fresh or stored at –20°C or –80°C (73 samples). All samples were run in duplicate. Absorption was determined with an ELISA reader at 450 nm (or 405 nm) against 690 or 620 nm as reference. The standard curve was linear between 2.2 and 600 pg/ml. Assay sensitivity was 2.8 pg/ml for OPG and 8 pg/ml for sRANKL, with intra-assay coefficients of variation <15% and for OPG with an inter-assay coefficient of variation <15%.

Statistical analyses of results were done using Statistica software, applying Mann-Whitney's test and non-parametric Spearman's correlation coefficient.

RESULTS

Figs. 1 and 2 illustrate serum OPG concentrations in individual MM patients and healthy subjects. In 20% of the MM patients, OPG levels were above 159 pg/ml, which was the highest level measured in the healthy subjects. The mean OPG serum level in the whole group of MM patients was 118±68 pg/ml (mean ±SD)

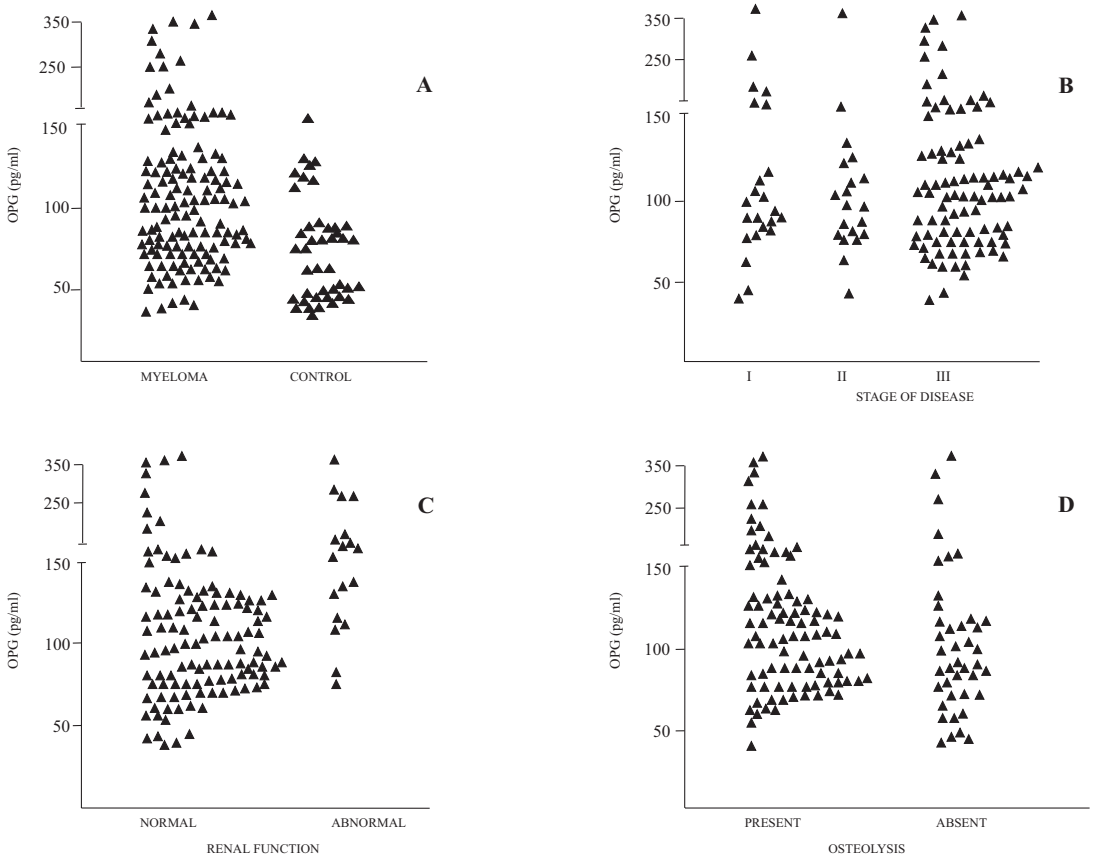

Figure 1. Serum OPG levels in particular patients with MM. Serum OPG levels in patients with myeloma and in healthy controls (**A**). Serum OPG levels in myeloma grouped by stage of disease (**B**). Serum OPG levels in myeloma grouped by renal function: normal – patients with serum creatinine level <176 μmol/l, abnormal – patients with serum creatinine level >176 μmol/l (**C**). Serum OPG levels in myeloma grouped by occurrence of osteolytic lesions (**D**).

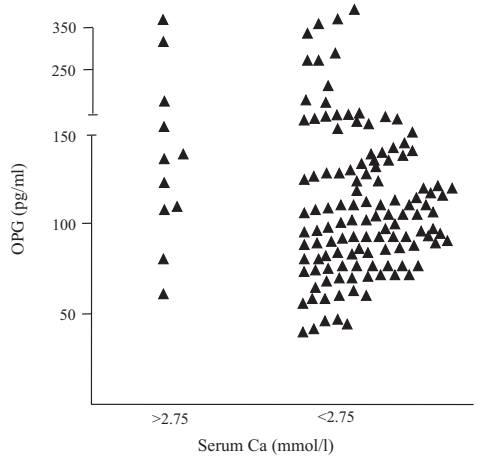

Figure 2. Serum OPG levels in individual patients with MM grouped by serum calcium (Ca) concentration

and in healthy age- and sex-matched controls 82±26 pg/ml. The median OPG concentrations in myeloma and control sera were 101 pg/ml (range: 40–445) and 81 pg/ml (range: 43–159), respectively. This difference was statistically significant ($p<0.001$).

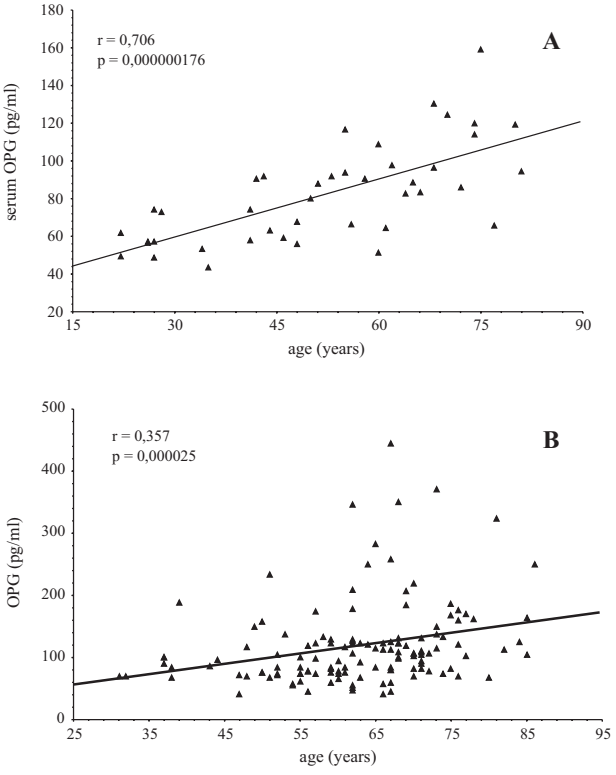

Figure 3. Positive correlation of serum concentration of OPG and age in healthy subjects (**A**) and in patients with MM (**B**).

Table 1. Serum osteoprotegerin level in healthy individuals and in MM patients in relation to gender, stage of disease, presence of osteolysis, hypercalcaemia, renal failure and monoclonal protein isotype

Study group	Number of cases	Osteoprotegerin concentrations (pg/ml)			p value
		mean ±SD	median	range	
Healthy persons	42	82±26	81	43–159	0.0001
MM patients	133	118±68	101	40–445	
Male	72	120±65	106	45–445	0.1225
Female	61	114±71	99	40–371	
Stage of disease					
I	24	124±87	92	42–445	I vs II 0.7236
II	20	109±64	96	45–350	I vs III 0.6890
III	89	119±64	106	40–371	II vs III 0.3956
Osteolysis					
present	92	120±63	105	40–371	0.1771
absent	41	114±79	87	42–445	
Hypercalcemia					
present	11	160±94	129	58–371	0.0458
absent	122	115±65	100	40–445	
Renal function					
normal	115	110±63	98	40–445	0.00005
abnormal	18	173±77	163	74–371	
M-protein					
IgG	82	114±65	98	40–350	IgG vs IgA 0.8008
IgA	32	102±40	96	45–219	IgG vs BJ 0.0071
Bence Jones (BJ)	13	182±113	157	74–445	IgA vs BJ 0.0071
IgD	2	132; 138			
IgM	1	112			
non-secretory	3	122; 122; 169			

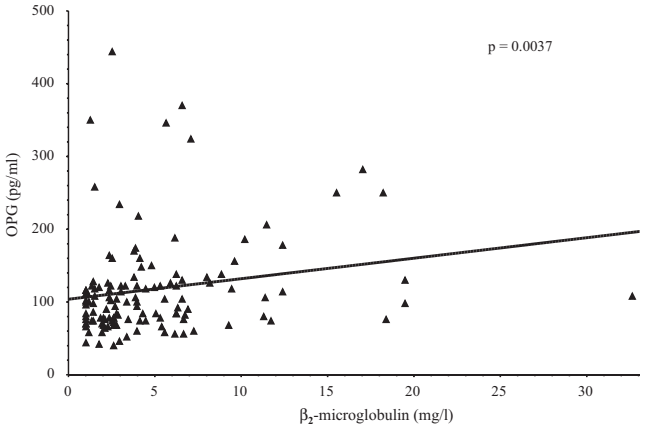

Figure 4. Positive correlation of serum concentration of OPG and β_2-microglobulin in patients with MM.

Results of serum OPG concentration in MM patients in relation to gender, stage of disease, presence of osteolysis, hypercalcemia, renal failure, and monoclonal protein isotype are presented in Table 1. OPG serum levels were higher in myeloma patients with renal failure than in patients with normal renal function ($p<0.001$; Fig. 1C). Similarly, OPG serum levels were higher in myeloma patients with hypercalcemia than in patients with normal serum calcium concentrations ($p=0.0458$; Fig. 2). Increased (>159 pg/ml) serum OPG level occurred with the same frequency in myeloma patients with osteolysis (17% of cases) as in those without it (17% of cases; Fig. 1D).

Serum level of OPG in healthy subjects increased significantly with age ($p=0.000000176$; Fig. 3A), as did those in MM patients ($p=0.000025$; Fig. 3B). There was a positive correlation between OPG and β_2-microglobulin serum concentrations ($p=0.0037$; Fig. 4). Among the 7 myeloma patients with increased OPG serum level above 250 pg/ml, 2 had renal failure, 1 immunoglobulin lightchain amyloidosis, and 1 generalized skin lesions with lymphocyte infiltration. Only in 2 of all the myeloma patients was the OPG level slightly below 43 pg/ml, i.e. the lowest level measured in the control group (Fig. 1A).

Figs. 5 and 6 illustrate serum sRANKL concentrations in particular MM patients and healthy individuals. Results of serum sRANKL concentrations in

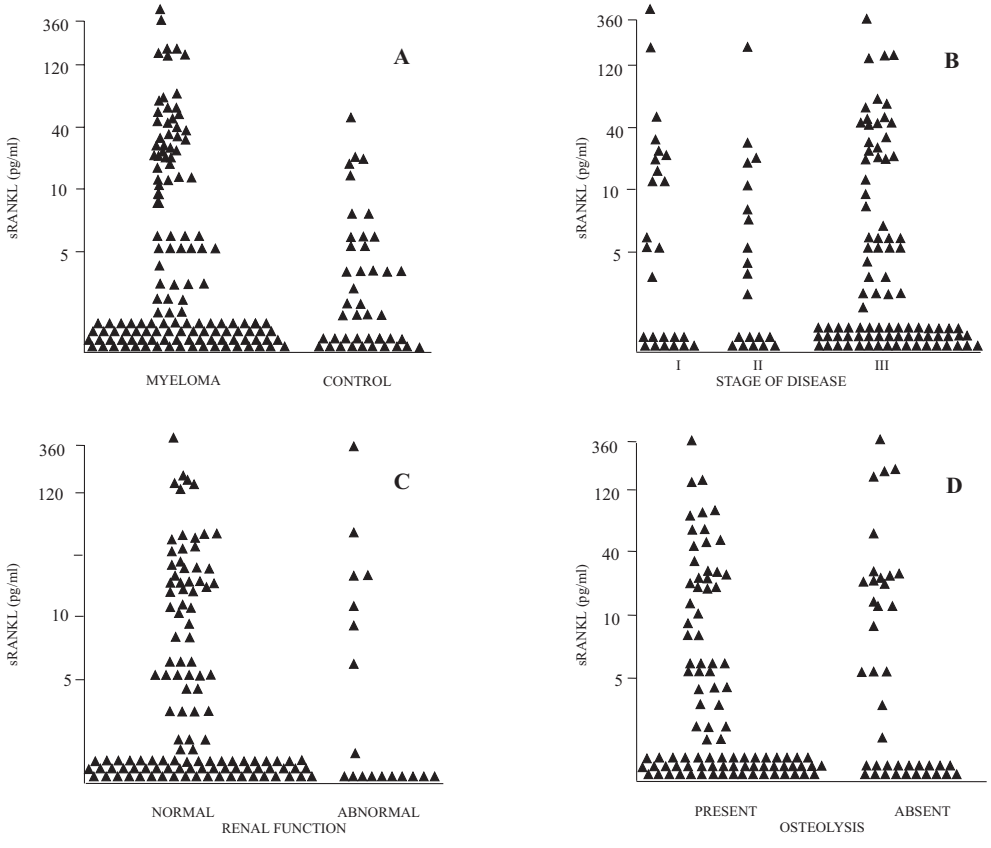

Figure 5. Serum sRANKL levels in particular patients with MM. **A** – serum sRANKL levels in patients with MM and in healthy controls; **B** – serum sRANKL levels in myeloma grouped by stage of disease; **C** – serum sRANKL levels in myeloma grouped by renal function: normal – patients with serum creatinine level <176 μmol/l, abnormal – patients with serum creatinine level >176 μmol/l; **D** – serum sRANKL levels in myeloma grouped by occurrence of osteolytic lesions.

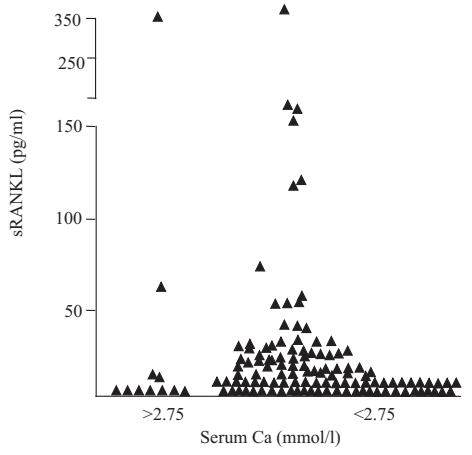

Figure 6. Serum sRANKL levels in individual patients with MM grouped by serum calcium concentration.

with a mean level of 128±76 and a median of 106 pg/ml, while BM OPG concentration ranged 40–397 pg/ml, with a mean level of 122.97±71.68 and a median of 103 pg/ml (p=0.502377). Serum sRANKL concentrations in individual patients ranged 0–293 pg/ml, with a mean level of 28±55 and a median of 8 pg/ml, while BM sRANKL concentration ranged 0–130 pg/ml, with a mean level of 24±26 and a median of 21 pg/ml (p=0.2823). There was a correlation between BM and serum OPG concentrations (p=0.0000000000013; Fig. 7A) and between BM and serum sRANKL concentrations (p=0.00000015; Fig. 7B). There was no correlation between BM OPG and sRANKL concentrations (p=0.3865) or between serum OPG and sRANKL concentrations (p=0.3286). Median values of the

Table 2. Serum sRANKL level in healthy individuals and in MM patients in relation to gender, stage of disease, presence of osteolysis, hypercalcemia, renal failure, and monoclonal protein isotype

Study group	Number of cases	RANKL concentrations (pg/ml)			p value
		mean ±SD	median	range	
Healthy persons	42	4.47±8.03	1	0–43	0.4186
MM patients	133	19.75±53.42	0	0–388	
Male	72	17.36±46.79	1	0–340	0.7011
Female	61	21.77±60.64	0	0–388	
Stage of disease					
I	24	32.79±84.32	5	0–388	I vs II 0.4436
II	20	14.65±40.97	2.5	0–185	I vs III 0.2812
III	89	17.10±44.69	0	0–340	II vs III 0.7541
Osteolysis					
present	92	15.25±41.83	0	0–340	0.4054
absent	41	29.62±73.80	2	0–388	
Hypercalcemia					
present	11	37.27±102.13	0	0–340	0.4401
absent	122	18.33±47.27	1	0–388	
Renal function					
normal	115	18.17±48.39	0	0–388	0.8719
abnormal	18	26.83±79.78	0	0–340	
M-protein					
IgG	82	10.34±21.85	0.5	0–152	IgG vs IgA 0.8130
IgA	32	33.96±81.01	0	0–388	IgG vs BJ 0.3601
Bence Jones (BJ)	13	23.30±37.08	5	0–127	IgA vs BJ 0.6164
IgD	2	0; 340			
IgM	1	2.0			
non-secretory	3	0; 0; 2			

MM patients in relation to gender, stage of disease, presence of osteolysis, hypercalcemia, renal failure and monoclonal protein isotype are presented in Table 2. In 45 MM patients the determinations of OPG and sRANKL concentration were performed in blood and BM samples collected simultaneously. In this group of MM patients, the serum OPG concentrations in individual patients ranged 42–445 pg/ml, sRANKL/OPG ratio for BM and serum of MM patients were 0.14 and 0.11, respectively. The differences in sRANKL/OPG level ratio depending on occurrence of osteolysis and stage of disease were not statistically significant (Table 3).

In 10 MM patients the determination of OPG and sRANKL serum concentrations was performed at the

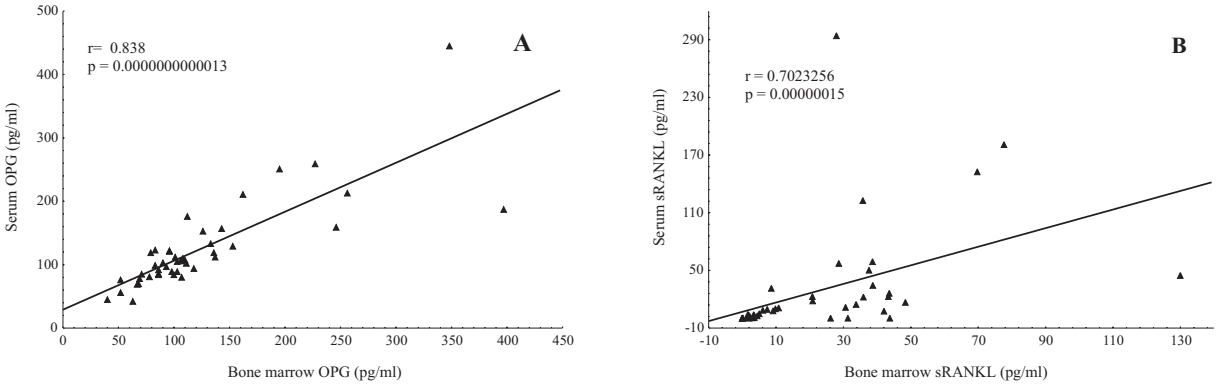

Figure 7. Positive correlation of serum and bone marrow OPG concentration in patients with MM (**A**). Positive correlation of serum and bone marrow sRANKL concentration in patients with MM (**B**).

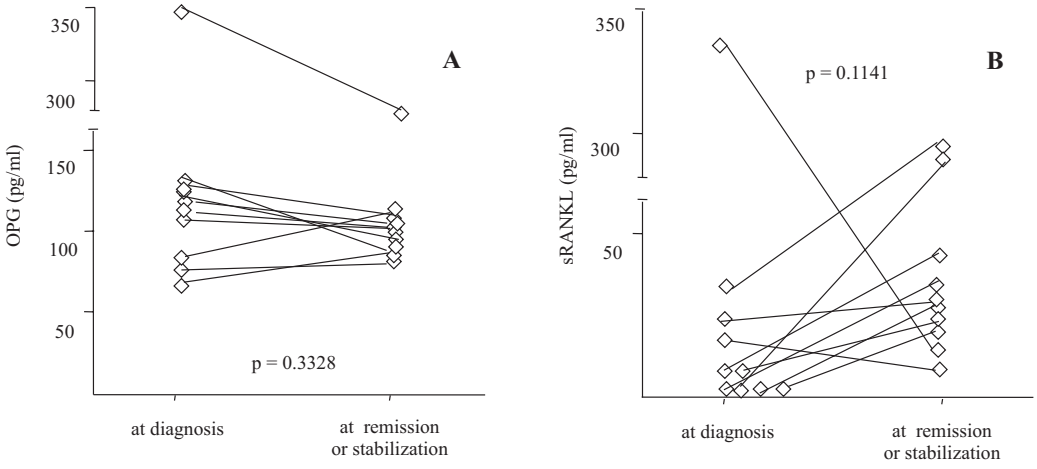

Figure 8. Serum OPG (**A**) and sRANKL (**B**) concentrations in individual MM patients at the time of diagnosis and in remission or stabilization of the proliferative process.

Table 3. Serum and bone marrow sRANKL/OPG concentration ratio in MM patients

Study group	N	Serum sRANKL/OPG ratio			p value	Bone marrow sRANKL/OPG ratio			p value
		mean ±SD	median	range		mean ±SD	median	range	
Healthy persons	42	0.35±0.50	0.11	0.01–1.74	0.0788				
MM patients	43	0.24±0.39	0.11	0–2.08		0.23±0.25	0.14	0–0.98	0.6440
Stage of disease									
I	11	0.36±0.65	0.12	0.02–2.06		0.33±0.33	0.20	0.03–0.91	
II	11	0.17±0.21	0.10	0.005–0.72	>0.4708	0.20±0.19	0.16	0.01–0.61	>0.4259
III	17	0.23±0.22	0.22	0.002–0.76		0.23±0.24	0.14	0.02–0.98	
Osteolysis									
present	28	0.21±0.31	0.11	0.002–1.38	0.0962	0.21±0.24	0.13	0.002–0.98	0.5067
absent	15	0.41±0.55	0.29	0.006–2.06		0.24±0.27	0.17	0.01–0.91	

time of MM diagnosis and also in the remission or stabilization of the proliferative process. Wilcoxon's pair test did not show differences depending on proliferative process phase both in OPG (p=0.3328) and sRANKL (p=0.1141) concentrations (Fig. 8). In 4 patients with plasma cell leukemia, serum OPG concentrations ranged 110–758 pg/ml (110; 122; 747, and 758 pg/ml) while serum sRANKL concentrations ranged 0–14 pg/ml (0; 0.3; 1, and 14 pg/ml). In 3 patients with MGUS, serum OPG concentrations ranged 79–95 pg/ml, while the serum sRANKL level was 0.67 pg/ml in 1 patient and was undetectable in 2 patients.

DISCUSSION

In our control group of healthy subjects, the serum OPG concentrations ranged 43–159 pg/ml and the arithmetic mean ±SD was 82±26 pg/ml. Our results are consistent with a study by Szulc et al.[49] conduced in 252 healthy men with an age range of 19–85 years in which the mean OPG concentration was 62±43 pg/ml. Similarly, in a study by Jung et al.[22] which included 36 male controls, the serum OPG concentrations were 7.1–74.4 ng/l, with a mean of 39±15 ng/l, and in a study by Politou et al.[42] which included 45 healthy individuals, the median serum OPG value was 116 pg/ml, with a range of 64–208 pg/ml. It should be noted that serum OPG concentrations of 5–130 ng/ml[16, 44] and 1–3 μg/l in healthy adults have also been reported[54]. OPG circulates in the peripheral blood in various forms: in complexes with soluble RANKL besides a free, unbound portion[19, 20]. The heterogeneity of these circulating forms may account for discrepancies in absolute values reported in the literature. Different available reagents recognize different OPG forms[9].

Studies designed to assess OPG serum levels in MM patients have yielded contradictory results[11, 16, 30, 32, 44]. Seidel et al.[44] reported the results of an analysis of OPG concentrations in 225 serum samples of MM patients at diagnosis who entered the Nordic Myeloma Study Group randomized α-interferon trial in the period from June 1990 to November 1992. OPG concentrations in particular patients ranged 2.6–80 ng/ml, with a mean concentration of 9.9±9 ng/ml and a median of 7.4 ng/ml, while in healthy age- and sex-matched controls (n=40), OPG levels ranged 5.1–130 ng/ml. In approximately 20% of the MM patients, OPG levels were below 5.1 ng/ml. In this study, long-term storage of myeloma serum samples could influence the results. In a study by Lipton et al.[32], OPG serum levels were 29% lower in patients with MM (n=34) than in healthy controls. In a study by Giuliani et al.[16], ELISA performed for MM patients (n=30) compared with a panel of 24 sex-matched healthy subjects also showed that OPG serum levels were reduced (mean ±SD: 18.5±6.0 ng/ml vs 26.6±4.8; p=0.009).

A recent study by Standal et al.[48] analyzed local OPG concentrations in plasma samples obtained from BM aspirates of 33 patients with MM and 27 healthy controls. In this study, OPG concentrations within the BM microenvironment were 27% lower in MM patients (median: 7.6 ng/ml, range: 1.0–77.6 ng/ml) than in healthy controls (median: 0.4 ng/ml, range: 5.3–200 ng/ml). Of note, OPG concentrations were 2-fold higher in BM plasma than in serum. The levels of OPG detected in the Giuliani et al.[16], Seidel et al.[44], and Standal et al.[48] panels of sera and BM were higher than those obtained by us and others[22, 30, 42, 49, 50], possibly because of the use of different antibodies to perform the ELISA. In a study by Politou et al.[42] which included 42 MM patients and 45 healthy controls, the MM serum OPG concentrations ranged 6–332 pg/ml, with a median of 94 pg/ml (=0.3–16.6 pmol/l and 4.7 pmol/l) and in controls the serum OPG concentrations were 64–208 pg/ml, with a median of 116 pg/ml (=3.2–10.4 pmol/l and 5.8 pmol/l).

The present study showed that serum OPG levels are not reduced in MM patients (mean: 118±68, median: 101 pg/ml, vs 82±26, median: 81 pg/ml, in healthy subjects; p=0.0001) and the values are higher in patients with renal failure (mean: 173±77, median: 163 pg/ml, vs 110±63, median: 98 pg/ml, in MM patients with normal renal function; p=0.00005). Positive correlations were found between serum concentrations of OPG and age (p=0.000025) and serum β_2-microglobulin concentrations (p=0.0037). We did not find any significant correlation either with the presence of osteolysis or with stage of disease. Our results confirm the observation of Kyrtsonis et al.[30], who found elevated OPG serum concentrations in MM patients (median 50 pg/ml vs 35 pg/ml in healthy subjects; p=0.03) which fluctuated depending on disease activity. Consistent with the present study, Corso et al.[11] have recently reported serum levels of OPG 34% higher in patients with MM (n=103) than in healthy controls (n=140), without significant correlation with the appearance of osteolysis. We found significant correlation between MM serum and BM OPG concentrations (Fig. 7A). Similarly, in a study by Standal et al.[48] the levels of OPG in the blood stream were found to be positively correlated with those in the BM plasma. These data seem to support the view that circulating OPG reflects its availability in the BM microenvironment.

The increase in the antiresorptive factor OPG in MM and its correlation with aging, both of which are associated with an increase in bone resorption, may reflect a protective mechanism of the skeleton to compensate for increased bone resorption and bone loss. OPG has been found to be overexpressed in bone metastases of prostate cancer patients. OPG inhibits prostate cancer-induced osteoclastogenesis and prevents prostate tumor growth in the bone[22, 56]. The results of study performed by Jung et al.[22] suggest that increased serum OPG is a marker of bone metastatic spread in prostate cancer patients. Our results show a significant increase in OPG with age in healthy females and males (Fig. 3A). An age-dependent increase in circulating serum concentrations of

OPG was also reported by Szulc et al.[49] Recently, Kudlacek et al.[29] reported influences of age and gender on serum levels of OPG in a precisely determined cohort of 1134 healthy subjects aged between 19 and 96 years at 17 Austrian outpatient bone clinics. The mean OPG serum level in all participants were 50.83±51.47 pg/ml (n=1134, median: 36, range: 2–584) and they observed a sharp increase in females after 60 years and in males after 70 years of age. OPG serum levels increased significantly with age, 2.1 pg/ml per year in females and 1.9 pg/ml per year in males ($p<0.0001$). In other studies, OPG concentrations in postmenopausal women with a high rate of bone turnover were higher than in those with a low rate of bone turnover; they were substantially higher in postmenopausal women with the most severe degrees of osteoporosis[13, 54]. The observation that in both sexes serum OPG normally increases with advancing age may be viewed as counter-evidence for disease-related mechanisms accounting for higher levels in MM patients. It is currently agreed that OPG gene is expressed by many cell types, so OPG levels may come from a number of sources[6]. Given the wide expression of OPG in human tissues, caution must be used in interpreting serum and BM OPG as a specific marker of myeloma bone disease[11].

The present study showed that serum sRANKL levels are not significantly increased in MM patients (mean: 19.75±53.42 pg/ml vs 4.47±8.03 pg/ml in healthy subjects; $p=0.4186$) and that there is significant correlation between MM serum and BM sRANKL concentrations (Fig. 7B). It also showed that sRANKL levels did not correlate with disease stage and skeletal manifestations (Table 2) and that the serum sRANKL/OPG ratio was similar in MM patients (median: 0.11, range: 0–2.08) and healthy individuals (median: 0.11, range: 0.01–1.74; $p=0.0788$; Table 3). Two studies have recently evaluated the role of sRANKL serum levels in myeloma bone disease[42, 50]. In the first study, Terpos et al.[50] measured serum sRANKL and OPG in 121 patients with MM diagnosed between 1992 and 2000 and in 45 controls. They found that serum sRANKL levels were higher in MM patients (median: 7.06 pM, range: 0.88–41.94 pM) than in control patients (median: 2.66 pM, range: 0.81–4.73 pM; $p<0.0001$). In this study, serum OPG levels were lower in MM patients (median: 4.71 pM, range: 0.31–16.66 pM) than in control patients (median: 7.98 pM, range 7.77–8.54 pM; $p=0.007$). The sRANKL/OPG ratio was increased in MM patients (median: 1.58, range: 0.10–44.4) compared with controls (median: 0.33, range: 0.10–0.59; $p<0.001$). They found that the sRANKL/OPG ratio can predict survival in MM. the aforementioned investigation[50] is not convincing because in that study the long-term storage of myeloma serum samples as well as methodological issues could influence the results. It should be noted that the same authors obtained quite different results in a study reported later[42] which included 42 MM patients and 45 healthy controls in whom the MM serum sRANKL concentrations ranged 0.07–1.85 pmol/l, with a median of 0.27 pmol/l (=1.4–37 pg/ml, median: 5.4 pg/ml) and in controls 0–3.95 pmol/l, with a median of 0.02 pmol/l (=0–79 pg/ml, median: 0.4 pg/ml). The median sRANKL/OPG ratio in MM patients' serum was 0.057 (0.024–2.64) and in controls 0.003 (0–1.23).

Moreb et al.[36] suggested the usefulness of OPG determinations in monitoring MM treatment. We did not find significant differences in OPG and sRANKL serum levels at diagnosis and in the plateau phase of disease (Fig. 8). In another study[11], increased serum OPG levels were noted at onset and under MM treatment. In our patients with MGUS, serum OPG concentrations were within normal limits and serum sRANKL was undetectable in 2 of the 3 investigated patients. Two studies have recently evaluated the role of OPG serum levels in MGUS[11, 42]. Corso et al.[11] found increased OPG serum levels in MGUS patients with respect to healthy controls while Politou et al.[42] did not find statistically significant differences between serum OPG concentrations in MGUS patients and healthy controls. Politou et al.[42] showed that serum sRANKL levels and the sRANKL/OPG ratio were increased in MGUS patients compared with healthy controls. In all our patients with plasma cell leukemia, serum sRANKL concentrations were within normal limits. The extremely high serum OPG levels (747 and 758 pg/ml) found in 2 of the 4 investigated patients were probably at least partly connected with renal failure in these patients. To the best of our knowledge, no data are available in the literature on OPG and sRANKL in plasma cell leukemia patients.

In conclusion, serum OPG levels are not reduced in MM patients; on the contrary, in 20% of patients the levels are elevated and this may be a compensative reaction related to increased bone destruction. Increased OPG concentrations in MM patients with renal failure may be related to its decreased elimination. The results of sRANKL concentration determinations using the kits produced by BIOMEDICA we[10, 26, 28] and others[42, 50] obtained need very scrupulous and careful assessment due to the observed differences in results coming from using various series of kits. Determinations of BM and the serum sRANKL/OPG concentration ratio seem to present no clinical value.

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