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Unusual Association between Increased Bone Resorption and Presence of Paroxysmal Nocturnal Hemoglobinuria Phenotype in Multiple Myeloma

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Abstract

Paroxysmal nocturnal hemoglobinuria (PNH) clones deficient in glycosylphosphatidylinositol-anchored molecules, including CD55 and CD59, have been previously described in patients with multiple myeloma (MM). The aim of this study was to investigate the possible association between existence of the PNH phenotype and myeloma bone disease. Forty-three patients with newly diagnosed MM were the subjects of the study. Radiographic evaluation of the skeleton was performed in all patients at diagnosis. The following biochemical markers were measured: bone resorption markers (tartrate-resistant acid phosphatase isoform 5b [TRACP-5b]and N-terminal cross-linking telopeptide of type-I collagen [NTX]), bone formation markers (bone alkaline phosphatase [bALP] and osteocalcin [OC]), osteoprotegerin (OPG), soluble receptor activator of nuclear factor κB ligand (sRANKL), and interleukin 6 (IL-6). Detection of CD55- and/or CD59-deficient red cell populations was performed after diagnosis. Patients with MM had elevated mean baseline NTX, TRACP-5b, sRANKL, and IL-6 levels compared with controls, whereas the mean values of bALP, OC, and OPG were significantly decreased. Four patients had no osteolytic lesions, whereas 8 patients had 1 to 3 lytic lesions, and 31 patients had more than 3 lytic lesions and/or pathologic fractures in the skeletal survey. CD55- and/or CD59-deficient red cell populations were observed in 56% of patients with MM. There was a strong correlation between the presence of PNH-like erythrocytes and increased bone resorption, as measured by NTX, TRACP-5b, and sRANKL/OPG ratio ($P < .03$, $P < .02$, and $P < .02$, respectively). There was also a significant correlation between PNH phenotype and severe bone disease ($P < .02$). These results suggest that there is a possible link between PNH phenotype and increased osteoclastic activity in MM owing to a potential effect of myeloma microenvironment on a preexisting PNH clone. Further studies are required for clarifying this phenomenon and investigating possible mechanisms of this unusual association. *Int J Hematol.* 2003;78:344-348.

Key words: Multiple myeloma; Paroxysmal nocturnal hemoglobinuria; Tartrate-resistant acid phosphatase isoform 5b (TRACP-5b); N-terminal cross-linking telopeptide of type I collagen (NTX); Soluble receptor activator of nuclear factor κB ligand (sRANKL)

1. Introduction

Bone involvement is a central feature of multiple myeloma (MM) and is characterized by increased osteoclastic activity not accompanied by a comparable increase in bone formation [1]. Bone resorption is mediated by cytokines, which are produced locally in the bone marrow microenvironment by cells of tumor or nontumor origin, including interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), and interleukin 11 (IL-11) [2]. The osteoprotegerin (OPG)/receptor activator of NF-κB (RANK)/RANK ligand (RANKL) system has been recognized as the dominant, final mediator of osteoclastogenesis. The level of OPG, which is the decoy receptor for RANKL, is decreased in MM patients, and myeloma cells affect the OPG/RANKL system, playing an important role in the pathogenesis of MM-induced bone disease [3-5]. Different biochemical bone

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markers have been used to reflect the extent of bone disease in MM. N-terminal cross-linking telopeptide of type I collagen (NTX) is considered to be one of the most sensitive bone resorption markers, whereas osteocalcin (OC) and bone-specific alkaline phosphatase (bALP) reflect bone formation [6]. Tartrate-resistant acid phosphatase isoform 5b (TRACP-5b), which is secreted by activated osteoclasts, is a novel marker of bone resorption and is considered to be a reliable marker for monitoring antiresorptive treatment in both osteoporosis and MM [7-9]. There are also data showing increased TRACP-5b levels in patients with MM and breast cancer with bone metastasis [9-11].

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired stem cell disorder characterized by intravascular hemolysis, thrombotic events, and bone marrow failure [12]. The characteristic defect in PNH is somatic mutation of the PIG-A gene, which is essential for biosynthesis of the glycosylphosphatidylinositol (GPI) anchor that affixes a number of proteins to the cellular surface [13,14]. Decay-accelerating factor (DAF) (CD55) and membrane inhibitor of reactive lysis (MIRL) (CD59) belong to the group of proteins that are linked to the cell membrane via the GPI anchor [15]. CD55 inhibits assembly of C3 and C5 convertases of the classic and alternative pathways and thereby it regulates the complement cascade at the C3 step. CD59 limits polymerization of C9 in membrane C5b-9 complex [16]. These molecules are distributed in all hematopoietic and other tissue cells, but they are not present in the blood cells of patients with PNH [13-15]. PNH clones with CD55 and/or CD59 deficiency have been reported to be present in different hematological disorders, mainly in aplastic anemia and myelodysplastic syndromes but also in lymphoproliferative disorders, including MM [17-21].

The aim of this study was to investigate possible correlations between the presence of the PNH phenotype in patients with MM and various biochemical parameters, including markers of bone remodeling, osteoclast function, and disease activity. Interactions between myeloma and stromal cells are crucial for the biology of MM, whereas the presence of the PNH phenotype in hematological malignancies may be immune mediated. We were especially interested in investigation of possible correlations between the presence of the PNH phenotype and levels of molecules involved in the pathogenesis of myeloma bone disease.

2. Patients and Methods

2.1. Patients

Forty-three patients with newly diagnosed MM (19 men, 24 women) were the subjects of the study. The median age was 65 years (range, 51-89 years). Two percent of the patients had disease in stage I, 16% in stage II, and 81% in stage III. The patient characteristics are shown in Table 1. A grading of bone morbidity in 3 stages according to radiographic evaluation of the skeleton was made (A, no osteolytic lesions or osteoporosis; B, 1-3 osteolytic lesions; C, >3 osteolytic lesions and/or pathologic fractures). The following biochemical markers were measured at diagnosis: bone resorption markers (TRACP-5b, NTX), bone formation markers (bALP, OC), OPG, soluble RANKL (sRANKL), IL-6, paraprotein, C-reactive protein (CRP), and β_2-microglobulin. These biochemical parameters also were measured in 45 age- and sex-matched healthy controls. CD55- and CD59-deficient red cell populations (PNH phenotype) were evaluated at diagnosis in all MM patients before administration of any antimyeloma treatment.

2.2. Methods

NTX values in the urine were determined by an enzyme-linked immunosorbent assay (ELISA, Osteomark NTX

Table 1.
Clinical Characteristics of Patients and Controls*

	Multiple Myeloma Patients	Controls
No. of subjects	43	45
Sex	19M, 24F	25M, 20F
Median age, y (range)	65 (51-89)	62 (50-74)
Type of multiple myeloma (no. of patients)	IgG (26), IgA (10), BJ (5), IgD (1), NS (1)	
Salmon-Durie stage (no. of patients)	I (1), IIA (6), IIB (1), IIIA (27), IIIB (8)	
Bone disease (no. of patients)	A (4), B (8), C (31)	
Mean hemoglobin, g/dL (range)	10.8 (8.7-14.1)	14.8 (13.2-16.4)
Mean paraprotein, g/L (SD)	31.7 (41.8)	—
Mean serum β_2-microglobulin, mg/L (SD)	3.9 (2.7)	1.4 (0.6)
Mean serum CRP, mg/L (SD)	9.7 (11.1)	2.3 (0.8)
Mean urine NTX, nM BCE/mM creatinine (SD)	194.2 (119.7)	34.2 (24.9)
Mean serum TRACP-5b, U/L (SD)	8.1 (5.9)	2.8 (2.3)
Mean serum sRANKL, pmol/L (SD)	6.9 (5.4)	2.1 (2.0)
Mean serum OPG, pmol/L (SD)	4.1 (3.5)	7.9 (0.7)
Mean sRANKL/OPG ratio (SD)	1.3 (14.6)	0.3 (0.3)
Mean serum bALP, U/L (SD)	14.3 (11.8)	33.6 (19.1)
Mean serum OC, ng/mL (SD)	11.3 (8.3)	37.9 (10.6)
Mean serum IL-6, pg/mL (SD)	31.0 (27.2)	2.1 (0.8)

*Ig indicates immunoglobulin; BJ, Bence-Jones; NS, nonsecretory; CRP, C-reactive protein; NTX, N-terminal cross-linking telopeptide of type I collagen; BCE, bone collagen equivalents; TRACP-5b, tartrate-resistant acid phosphatase isoform 5b; sRANKL, soluble receptor activator of nuclear factor κB ligand; OPG, osteoprotegerin; bALP, bone alkaline phosphatase; OC, osteocalcin; IL-6, interleukin 6.

urine; Ostex International, Seattle, WA, USA) that had intraassay and interassay coefficients of variation (CVs) of 7.6% and 4%, respectively. The normal values ranged between 20 and 55 nM bone collagen equivalents (BCE)/mM creatinine (to 75 nM BCE/mM creatinine for postmenopausal women). Serum TRACP-5b was measured with a solid-phase immunofixed enzyme activity assay (Bone TRAP assay; SBA, Oulu, Finland). The sensitivity of the assay is 0.06 U/L; intraassay and interassay CVs are less than 6% and 8%. Normal values ranged from 0.5 to 3.8 U/L for men and premenopausal women and up to 4.8 U/L for postmenopausal women. OPG and sRANKL also were measured by ELISA methods (Biomedica Gesellschaft, Vienna, Austria) that have intraassay and interassay CVs of less than 10%. Bone ALP was determined by ELISA methodology (Metra BAP EIA kit; Quidel, San Diego, CA, USA). The sensitivity of the assay is 0.7 U/L; intraassay and interassay CVs are less than 6% and 8%. Normal values ranged from 11.6 to 42.7 U/L. An ELISA assay also was used to determine the values of OC (N/MID Osteocalcin; Osteometer BioTech, Herley, Denmark) (normal value for men, 11-46 ng/mL; normal value for postmenopausal women, 25-48 ng/mL), IL-6 (Genzyme Diagnostics, San Carlos, CA, USA) (normal value, <3 pg/mL), and β_2-microglobulin (IMx system; Abbott Laboratories, Abbott Park, IL, USA) (normal value, 1.2-2.1 mg/L). The Sephacryl gel microtyping system was used for detection of CD55- and CD59-deficient red cell populations (DiaMed-ID Micro Typing System PNH test; DiaMed, Cressier sur Morat, Switzerland), as described previously [22].

2.3. Statistical Analysis

Statistical analysis was performed with the statistical package SPSS version 8.00. Associations between bone disease stage and biochemical markers were examined by the Kruskal-Wallis test. The Spearman rank correlation test was used to examine relationships between various parameters and clinical patient characteristics. Differences between patients and controls were evaluated with the Wilcoxon signed rank sum test. All *P* values were 2 sided, and confidence intervals referred to 95% boundaries.

3. Results

Patients with MM had elevated mean baseline NTX, TRACP-5b, sRANKL, and IL-6 values compared with controls ($P < .001$, $P < .001$, $P < .02$, and $P < .001$, respectively). The mean values of bALP, OC, and OPG were decreased compared with control values ($P < .001$, $P < .01$, and $P < .02$, respectively) (Table 1). NTX levels were above the upper normal limit in 28 (65%) of 43 patients, whereas abnormal values of TRACP-5b were observed in 31 (72%) of 43 MM patients. In 22 (51%) of the patients, OPG levels were below 7.7 pmol/L, the lowest level measured in the control group. In 30 (69%) of the patients sRANKL levels were above 4.7 pmol/L, the highest level measured in the control group. Finally, 33 (76%) of the patients had an sRANKL/OPG ratio above 0.59, which was the highest value observed in the control group. Four patients had no osteolytic lesions at baseline (group A), whereas 8 patients had 1 to 3 lytic lesions (group B), and 31 patients had more than 3 lesions and/or pathologic fractures in the skeletal survey (group C).

CD55- and/or CD59-deficient red cell populations were observed in almost 56% of patients with MM (Table 2). Four (9%) of the patients had concomitant deficiency of CD55 and CD59, and 20 (46%) of the patients had isolated deficiency of CD55. No patient was found to have isolated deficiency of CD59. The populations of cells with CD55 and/or CD59 deficiency never exceeded 10% of the total red cell population. No patient showed any clinical or laboratory sign of hemolysis.

There was strong correlation between the presence of CD55- and/or CD59-deficient erythrocytes and increased bone resorption, as defined by elevated levels of NTX and TRACP-5b and increased sRANKL/OPG ratios (Table 3). There also was significant correlation between the presence of PNH phenotype and the presence of severe bone disease (group C) in the skeletal survey ($P = .012$). No association was observed between existence of PNH clone and IL-6, CRP, paraprotein, or β_2-microglobulin values. The association between presence of PNH phenotype and different parameters is depicted in Table 3.

4. Discussion

We describe an unusual relationship between the presence of PNH-type red cells and increased bone resorption in patients with MM. PNH clones deficient in GPI-anchored molecules have been described in different hematological malignancies [17-24]. Our group has reported that patients with MM have erythrocytes with CD55 and/or CD59 deficiency at a proportion between 50% and 60% [20]. In this study red cells with a PNH-like defect were observed in almost 56% of patients with MM. This study is the first in which an association between existence of PNH phenotype and clinical features of the disease has been reported in MM.

Table 2.
CD55- and CD59-Deficient Red Cell Populations in Patients with Multiple Myeloma*

Diagnosis, n	$CD55^-/CD59^-$, n (%)	$CD55^-/CD59^+$, n (%)	$CD55^+/CD59^-$, n (%)
Multiple myeloma	4/43 (9.3%)	20/43 (46.5%)	0/43
IgG (26)	2/26 (7.6%)	14/26 (53.8%)	0/26
IgA (10)	1/10 (10%)	4/10 (40%)	0/10
BJ (5)	0/5	2/5 (40%)	0/5
IgD (1)	1/1	0/1	0/1
NS (1)	0/1	0/1	0/1

*Ig indicates immunoglobulin; BJ, Bence-Jones; NS, nonsecretory.

Table 3.
Correlation between Presence of CD55 and/or CD59 Red Cell Deficiency and Bone Resorption*

Parameter (n)	CD55 and/or CD59 Deficiency, n (%)	*P*
Increased NTX (28)	19/28 (67.8)	.024
Normal NTX (15)	5/15 (33.3)	
Increased TRACP-5b (31)	22/31 (70.9)	.016
Normal TRACP-5b (12)	2/12 (16.6)	
Increased sRANKL/OPG ratio (33)	22/33 (66.6)	.012
Normal ratio (10)	2/10 (20)	
Bone disease, groups A + B (12)	1/12 (8.3)	.012
Bone disease, group C (31)	23/31 (74.1)	
IL-6 ≤10 pg/mL (14)	8/14 (57.1)	.876
IL-6 >10 pg/mL (29)	16/29 (55.1)	
IL-6 ≤20 pg/mL (18)	10/18 (55.5)	.992
IL-6 >20 pg/mL (25)	14/25 (56)	
CRP ≤8 mg/L (17)	9/17 (52.9)	.728
CRP >8 mg/L (26)	15/26 (57.6)	
β_2-Microglobulin ≤3 mg/L (16)	11/16 (68.7)	.381
β_2-Microglobulin >3 mg/L (27)	13/27 (48.1)	
Paraprotein ≤30 g/L† (11)	7/11 (63.6)	.412
Paraprotein >30g/L (25)	14/25 (56)	

*NTX indicates N-terminal cross-linking telopeptide of type I collagen; TRACP-5b, tartrate-resistant acid phosphatase isoform 5b; sRANKL, soluble receptor activator of nuclear factor κB ligand; OPG, osteoprotegerin; IL-6, interleukin 6; CRP, C-reactive protein.

†For IgG and IgA myeloma patients.

Myeloma bone disease includes mainly osteolytic lesions due to unbalanced bone turnover with an increased resorptive phase [1]. Activation of osteoclasts through the RANK/RANKL/OPG pathway plays an important role in the pathogenesis of osteolytic lesions in MM [4,5]. In vitro studies have shown that the increased ratio of RANKL/OPG in MM is the dominant, final mediator of osteoclastogenesis and is associated with increased osteoclast activation [25]. RANKL is mainly produced by osteoblasts and marrow stromal cells as a membrane-bound protein. It subsequently is cleaved into a soluble form (sRANKL) by the metalloprotease-disintegrin TNF-α–converting enzyme [26]. Myeloma cells induce RANKL expression by bone marrow stromal cells, a process that leads to increased osteoclastic activity, increased bone resorption, and, more important, development of osteolytic lesions [4,26]. Furthermore, myeloma cells inhibit production and induce degradation of OPG; the result is reduced OPG levels in the serum of MM patients [3,4,27,28]. Therefore there is an increased RANKL/OPG ratio in MM, which induces formation and activation of osteoclasts. In this study we confirmed that at diagnosis patients with MM have increased levels of sRANKL and decreased levels of serum OPG. This condition leads to increased osteoclast activation and bone resorption, as measured by TRACP-5b and NTX levels. Osteoclast activation also is mediated by cytokines, such as IL-6 and TNF-α [1]. Increased serum levels of IL-6 have been reported in MM [29,30]. In this study the levels of IL-6 in MM patients were increased compared with control values.

The presence of red cells with the PNH phenotype may be due to a new mutation in the myeloma clone affecting the PIG-A gene or the genes encoding CD55 and CD59, as has been described for acute leukemia [31]. However, the myeloma bone marrow microenvironment and overexpression of different cytokines (IL-6, TNF-α, IL-11, OPG, RANKL) may offer a survival advantage, in a preexisting population with CD55 and CD59 deficiency that can proliferate and be detected. The presence of extremely low levels of GPI-deficient neutrophils or erythrocytes in healthy individuals, as well as existence of PNH-like clones in a very small proportion of cells prior to selection in their favor by anti-CD52 (Campath-1H) administration in patients with chronic lymphocytic leukemia, supports the second hypothesis [32-34]. The strong correlation between sRANKL/OPG ratio, which is crucial for osteoclast growth and differentiation, and the presence of PNH phenotype suggests that the myeloma bone marrow microenvironment may create suitable conditions for a preexisting PNH clone to proliferate. Conversely, we found no correlation between the existence of PNH phenotype in MM and serum levels of IL-6, another important cytokine for development of myeloma bone disease. Furthermore, sRANKL/OPG ratio has been reported to correlate with IL-6 serum levels [4]. A larger number of patients and measurements of IL-6 levels in the bone marrow plasma may be required to draw final conclusions about the correlation between PNH phenotype and IL-6. However, the high percentage of PNH phenotype in patients with severe bone disease but not in patients with high paraprotein levels indicates that the myeloma microenvironment in patients with increased osteoclastic activity may influence the growth of a possible preexisting PNH clone. This hypothesis, however, remains to be proven.

In this study, the proportion of red cells with CD55 and/or CD59 deficiency never exceeded 10% of the total erythrocytic population. This phenomenon may support the hypothesis of the preexisting clone. On the other hand, expression of CD55 is controlled mainly by transcriptional regulation. This phenomenon leads to the hypothesis that a possible mutation in the myeloma clone may affect the presence of CD55 in the cell membrane, because the majority of patients had isolated deficiency of CD55 [31].

This study revealed an unusual association between presence of the PNH phenotype and increased bone resorption in patients with MM. Further studies and larger numbers of patients are needed to confirm this finding and to investigate the possible underlying mechanisms of this relationship.

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