Karyotypic Analysis of Bone Marrow Cells in Pyodermic Lesions Associated With Myelodysplastic Syndrome

Toshihisa Hamada, MD; Hironori Matsuura, MD; Takashi Oono, MD; Shin Morizane, MD; Osamu Yamasaki, MD; Kenji Asagoe, MD; Takenobu Yamamoto, MD; Kazuhide Tsuji, MD; Keiji Iwatsuki, MD

Background: Recalcitrant pyodermic lesions and neutrophilic dermatoses are often associated with subclinical myelodysplastic syndrome (MDS). In this case series, we assessed the diagnostic importance of karyotypic analysis of bone marrow cells in 4 patients with MDS-associated pyodermic eruptions treated at our university hospital. Karyotypic analysis was performed in bone marrow cells and peripheral blood lymphocytes obtained. Serum levels of granulocyte colony-stimulating factor were measured.

Observations: Four patients with pyodermic eruptions or neutrophilic dermatosis had chromosomal abnormalities in bone marrow cells, including del(20)(q11;q13.3) in 2 patients, trisomy 8 in 1 patient, and t(11;22)(q23;q11) in 1 patient. Three patients without morphologic findings suggestive of MDS were diagnosed as having refractory anemia. One female patient had refractory anemia with ringed sideroblasts associated with del(20). Two patients with refractory anemia had a normal karyotype in peripheral blood lymphocytes. Two patients with elevated serum levels of granulocyte colony-stimulating factor had more active or widespread cutaneous diseases.

Conclusions: Karyotypic analysis of bone marrow cells, but not of peripheral blood lymphocytes, is essential in proving a diagnosis of MDS-associated pyodermic lesions. The overexpression of granulocyte colony-stimulating factor, which may compensate for impaired hematopoiesis in patients with MDS, seems to be a key cytokine leading to neutrophilic infiltration.

Arch Dermatol. 2008;144(5):643-648

Neutrophilic dermatosis (ND) skin diseases are characterized by aseptic dermal neutrophilic infiltrates, which include pyoderma gangrenosum, acute febrile ND, and other pyodermic eruptions. Because these diseases are often associated with myelodysplastic syndrome (MDS), hematologic examination is required to search for the presence of MDS. However, routine blood test results and bone marrow findings are sometimes insufficient to confirm subclinical MDS. Myelodysplastic syndrome constitutes a group of clonal hematopoietic stem cell diseases characterized by dysplasia and ineffective hematopoiesis in 1 myeloid cell lineage or more. Therefore, it is important to detect abnormal hematopoietic stem cells by cytogenetic analysis of bone marrow cells, especially in MDS types such as refractory anemia and refractory anemia with ringed sideroblasts and unilineage dysplasia, in which dysplasia is confined to the erythroid lineage.

Because patients with ND often have elevated serum levels of granulocyte colony-stimulating factor (G-CSF), G-CSF-activated neutrophils may play a pivotal role in the development of ND. In cases of MDS-related ND, the overexpression of G-CSF may be related to compensatory hematopoiesis from normal hematopoietic stem cell lineages. Therefore, it is important to evaluate the pathogenic significance of G-CSF in the development of MDS-related ND.

We describe herein 4 patients with recalcitrant pyoderma associated with MDS, the diagnosis of which was confirmed by karyotypic analysis of bone marrow cells. Together with the serum G-CSF level findings, we discuss a pathogenic link between hematopoietic events in the bone marrow of patients with MDS and the occurrence of ND.

Case 1

A 33-year-old man had a 6-year history of recurrent pyoderma on the trunk, extremities, and other non-MDS-related locations. Two patients with refractory anemia had a normal karyotype in peripheral blood lymphocytes. Two patients with elevated serum levels of granulocyte colony-stimulating factor had more active or widespread cutaneous diseases.
ties, and head, as well as multiple verrucous lesions on the hands. The patient had a low-grade fever and had experienced weight loss and general malaise without gastrointestinal or articular symptoms. Peripheral neutropenia had been diagnosed when the patient was 12 years old, but no further examination was performed. His family history was not contributory. On physical examination, the patient had acneic eruptions on the face and neck (Figure 1) and pyodermic lesions and scar formation on the nape, axillae, and sacral area. The differential diagnosis included cystic acne, hidradenitis suppurativa, pyoderma gangrenosum, and pilonidal sinus. Except for coagulase-negative staphylococci, no pathogenic microbes were isolated from the lesions. Multiple verrucae were observed on the fingers and trunk, and some of them showed brownish plaque lesions suggestive of human papilloma virus–related Bowen disease.

Biopsy specimens from the pyodermic lesions revealed dense infiltration of neutrophils and mononuclear cells in the entire dermis, showing chronic abscess formation. No abnormal cell types were found in the dermal infiltrates by routine immunohistochemical analysis. In addition to these findings, sinus formation was observed in the axillary lesions, and parts of the epithelial sinus walls were composed of atypical keratinocytes. The verrucous lesions consisted of acanthotic epidermis with many vacuolated granular cells, and a brownish plaque lesion represented hyperkeratotic acanthosis composed of many atypical keratinocytes and dyskeratotic cells. Human papilloma virus 33 DNA was detected by polymerase chain reaction from both types of lesions.

Routine laboratory test results revealed a white blood cell count of 7400/µL (to convert white blood cell count to $10^9/L$, multiply by 0.001) (with 81% neutrophils, 18% lymphocytes, and 1% monocytes), a red blood cell count of 3.92 $10^12/µL$ (to convert red blood cell count to $10^12/L$, multiply by 1.0), a platelet count of 271 $10^3/µL$ (to convert platelet count to $10^9/L$, multiply by 1.0), and a hemoglobin level of 10.8 g/dL (to convert hemoglobin level to grams per liter, multiply by 10.0). Blood chemistry test results showed an elevated serum level of C-reactive protein at 3.4 mg/dL (to convert C-reactive protein level to nanomoles per liter, multiply by 9.524), and a decreased albumin level at 3.37 g/dL (to convert albumin level to grams per liter, multiply by 10.0). No abnormal findings were observed in the serum lactate dehydrogenase or immunoglobulin levels, anti–human immunodeficiency virus antibody, or CD4/CD8 ratio of peripheral blood lymphocytes. Thymidine uptake by lectin-stimulated lymphocytes was slightly decreased. The following serum cytokine levels were slightly elevated: G-CSF (27 pg/mL [reference range, 4.7–18.1 pg/mL]), interleukin 6 (11.1 pg/mL [reference range, <4 pg/mL]), and interleukin 8 (20.9 pg/mL [reference range, <20 pg/mL]). Computed tomography revealed the presence of mild lung fibrosis, reflecting the increased lev-

Figure 1. Case 1. A, Cystic acne on the front of the neck. B, Cystic acne and scar formation on the nape and occipital region. C, Chronic pyodermic plaque and scar formation on the right axilla.
els of sialylated carbohydrate antigen KL-6 at 668 U/mL (309 [157]). No abnormality was found by gastrointestinal or colonic fiberoptic examination. Gallium 67 citrate scintigraphy demonstrated mild accumulation of the isotopes in the elbow joints.

Bone marrow aspiration demonstrated no abnormality in the nuclear cell count or morphologic structure, except for the presence of a few abnormal megakaryocytes. G-banding chromosome analysis revealed trisomy 8 in 19 of 20 bone marrow cells (Figure 2), whereas a normal 46,XY karyotype was detected in 9 mitotic cells in the patient’s peripheral blood lymphocytes.

**CASE 2**

A 50-year-old man was seen with rapidly progressing eruptions on the face and a high-grade fever. In October 2002, the patient had had several pyodermic eruptions on the face, associated with headache. A similar eruption had occurred on the left side of the chest with tenderness and became granulomatous. His medical history was not contributory. One month before the first visit, erythremic plaques had developed on the face and spread to the trunk and extremities, associated with fever and arthralgia on the back. On physical examination, the patient had exudative erythremic plaques on the face, trunk, and extremities (Figure 3), and some of the eruptions were erosive. A biopsy specimen from an erythremic plaque showed infiltration of numerous neutrophils and marked edema in the dermis, without atypical cell infiltration.

Total blood cell counts showed a white blood cell count of 8400/µL (with 66% neutrophils, 13% lymphocytes, and 9% eosinophils), a red blood cell count of 4.12 × 10^6/µL, a hemoglobin level of 13.4 g/dL, and a platelet count of 250 × 10^3/µL. Blood chemistry levels were as follows: total protein, 7.67 g/dL (to convert total protein level to grams per liter, multiply by 10.0); albumin, 3.76 g/dL (to convert albumin level to grams per liter, multiply by 10.0); lactate dehydrogenase, 263 U/L (to convert lactate dehydrogenase level to microkatal per liter, multiply by 0.0167); and C-reactive protein, 4.0 mg/dL. Serum immunoglobulin levels were normal except for an elevated IgA level at 565.9 mg/dL (to convert IgA level to milligrams per liter, multiply by 10) without dysproteinemia. The CD4/CD8 ratio in the peripheral blood was 0.8 (references range, 0.9-3.2). Although neutrophil functions determined by phagocyte and killing activity were in the normal range, the G-CSF serum level was markedly increased at 59 pg/mL (reference range, 4.7-18.1 pg/mL).

Neither radiologic nor endoscopic examination revealed inflammatory bowel disease. Bone marrow aspiration demonstrated normal nuclear cell number and myelogram findings. However, chromosome analysis showed an abnormal karyotype of 46,XY,del(20)(q11;q13.3) in all 20 mitotic cells examined. The same abnormal karyotype was detected in 2 of 2 mitotic cells induced by lectin in the peripheral blood lymphocytes.

**CASE 3**

A 31-year-old man was referred to us because of acneic eruptions on the trunk and pyoderma on the buttocks. The patient had previously visited our orthopedic clinic for examination of osteitis on the right femoral bone and lumbago. No gastrointestinal symptoms were described. The patient was examined under a tentative diagnosis of SAPHO (synovitis, acne, pustulosis, hyperostosis, and osteomyelitis) syndrome.

On physical examination, multiple discrete pustules and papules were present on the chest and back. Pyodermic nodules and plaques were observed on the buttocks (Figure 4). In addition to these lesions, many depressive scars were present. These clinical features were consistent with those of chronic pyoderma gluteale. A biopsy specimen from the nodule showed dense infiltration of neutrophils in the upper dermis. Routine hematologic test results revealed a white blood cell count of 8800/µL with normal hemogram, a red blood cell count of 3.80 × 10^6/µL, a hemoglobin level of 11.6 g/dL, and a platelet count of 490 × 10^3/µL. No abnormalities were found in his serum total protein level, liver profile (including transaminases and bilirubin), or renal profile, except for a decreased serum level of lactate dehydrogenase at 107 U/L. His C-reactive protein level was increased to 7.6 mg/mL.

An osteolytic change in the right femoral bone was identified by computed tomography and was confirmed by bone...
biopsy. A high isotope accumulation was observed in the same lesion by bone scintigraphy using technetium.

Despite a normal nuclear cell count and myelogram, the chromosome analysis demonstrated the presence of 46,XY,t(11;22)(q23; q11) or t(11;22)(q24;q12) in all 20 mitotic cells in the bone marrow. No chromosomal abnormality was detected in the peripheral blood lymphocytes.

CASE 4

A 23-year-old woman with anemia and thrombocytopenia was referred to us because of pyodermic nodules on the face. The patient’s anemia had been identified 10 years earlier, and sideroblastic anemia related to MDS had been previously diagnosed. The patient’s medical and family history was unremarkable. Neither gastrointestinal symptoms nor arthralgia was reported.

Granulomatous pyodermic nodules, ranging from 0.5 to 2.0 cm in diameter, were found on the eyelids, and a brown scar was present on the right side of the forehead (Figure 4). A biopsy from the pyodermic nodule showed infiltration of neutrophils and mononuclear cells in the entire dermis without leukemic cells.

A peripheral blood cell count revealed a white blood cell count of 5800/µL (with a +1% decrease in neutrophils and a 57% relative increase in lymphocytes), a red blood cell count of 2.06 × 10^6/µL, a hemoglobin level of 7.0 g/dL, and a platelet count of 135 × 10^3/µL. The free iron level in serum was 198 µg/dL (to convert iron level to micromoles per liter, multiply by 0.179), with a total iron-binding capacity of 208 µg/dL and a ferritin level of 221.0 ng/mL (to convert ferritin level to picomoles per liter, multiply by 2.247). Blood chemistry tests showed normal results for liver profile and for total protein, albumin, and C-reactive protein levels, with an increased serum level of lactate dehydrogenase at 499 U/L.

Bone marrow aspiration revealed the presence of sideroblasts and an abnormal karyotype of 46,XX,del(20)(q11;q13.3) in all 20 mitotic cells examined. No abnormal karyotype was detected in 5 of 5 mitotic cells induced by lectin in the peripheral blood lymphocytes.

CYTOGENETIC ANALYSIS OF BONE MARROW CELLS

On morphologic examination of bone marrow cells, an excess number of ringed sideroblasts was detected in case 4, whereas no abnormalities were found in the other cases. Karyotypic examination demonstrated chromosomal abnormalities in all patients, including del(20)(q11;q13.3) in patients 2 and 4, trisomy 8 in patient 1, and t(11;22)(q23;q11) in patient 3. In patient 2, the same abnormal karyotype in the bone marrow cells was detected in the peripheral blood lymphocytes, but no abnormal karyotype was found in the peripheral blood lymphocytes of the remaining 3 cases. No patient had a history of the administration of carcinogenic agents or G-CSF. Based on these hematologic and cytogenetic findings, patients 1 through 3 (all males) were diagnosed as having refractory anemia, and patient 4 (female) was diagnosed as having refractory anemia with ringed sideroblasts.
Our patients had various types of ND, including cystic acne–like pyoderma (in cases 1 through 3), chronic pyoderma gangrenosum (in cases 1, 2, and 4), chronic glutéal pyoderma (in case 3), and acute febrile neutrophilic dermatosis–like manifestations (in case 2). Patients 1 and 2 with refractory anemia had severe systemic inflammatory symptoms such as fever and general malaise without clinically apparent MDS. These patients had elevated serum levels of G-CSF (27 pg/mL in case 1 and 59 pg/mL in case 2) associated with elevated levels of C-reactive protein. Patients 3 and 4, with normal serum levels of G-CSF, had less remarkable systemic symptoms and cutaneous diseases on limited regions. Ankylosing spondylitis was present in patients 1 through 3. Cutaneous lesions and articular symptoms responded well to systemic or topical injection of corticosteroid therapy.

**COMMENT**

Myelodysplastic syndrome is a heterogeneous group of clonal stem cell disorders characterized by dysfunction of hematopoiesis. Disturbance of hematopoietic cell growth and maturation leads to bone marrow failure with chronic or progressive peripheral cytopenias. About half (40%-70%) of the patients with primary MDS demonstrate cytogenetic abnormality in hematopoietic cell lines. The early stages of MDS are defined by excessive apoptosis of progenitor cells, which leads to ineffective hematopoiesis and is counterbalanced by increased proliferation of hematopoietic elements (Figure 5). Therefore, a paradoxical finding of peripheral cytopenias and normocellularity or hypercellularity in the bone marrow may occur in patients with MDS.

Our patients had chromosomal abnormalities in bone marrow cells, including trisomy 8 in case 1, del(20) in cases 2 and 4, and t(11;22) in case 3. Of the cytogenetic subtypes, del(20) chromosomal abnormality and trisomy 8 have been reported in 2% and 5% of patients with MDS, respectively. Patients 1 through 3 had few clinical and hematologic findings suggestive of MDS. These observations indicate that, despite the absence of apparent hematologic abnormalities, we should perform cytogenetic analysis of bone marrow cells in patients with recalcitrant pyodermic lesions or ND. Furthermore, it is notable that patients 1, 3, and 4 had a normal karyotype in peripheral blood lymphocytes. Therefore, bone marrow cells should be used to perform chromosomal analysis. Because patients with mild MDS such as refractory anemia and refractory anemia with ringed sideroblasts usually have unilineage dysplasia affecting the ery-
of hematopoietic signals, including G-CSF, may alter neutrophilic functions, resulting in the development of ND.

In conclusion, our observations indicate the importance of karyotypic analysis of bone marrow cells, but not of peripheral blood lymphocytes, in proving a diagnosis of MDS-associated pyodermic eruptions, despite the absence of overt hematologic abnormalities.

Accepted for Publication: June 24, 2007.

Correspondence: Keiji Iwatsuki, MD, Department of Dermatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama 700-8558, Japan (keijiiwa@cc.okayama-u.ac.jp).

Author Contributions: Dr Iwatsuki had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Hamada and Iwatsuki. Acquisition of data: Hamada, Matsuura, Oono, Morizane, Asagoe, and Yamamoto. Analysis and interpretation of data: Yamasaki and Tsuji. Drafting of the manuscript: Hamada and Iwatsuki. Administrative, technical, and material support: Asagoe and Iwatsuki. Study supervision: Iwatsuki.

Financial Disclosure: None reported.

REFERENCES