# A Retrospective Analysis of Cytogenetic and Clinical Characteristics in Patients With Multiple Myeloma

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**Abstract:** *Background:* Cytogenetic alterations in patients with multiple myeloma (MM) represent important risk factors in terms of prognosis. In this study, the impact of the cytogenetic aberrations of MM on patient clinical features and outcome was investigated. *Methods:* Conventional cytogenetic analysis with R-banding technique and molecular cytogenetic characterization by interphase fluorescence *in situ* hybridization (FISH) were used to detect aberrant chromosomal arrangements, including *17p13* and *13q14* deletions, *14q32* rearrangement and *1q21* amplification, in bone marrow nucleated cells from 65 patients. *Results:* About 16.9% of patients showed aberrations by conventional cytogenetic analysis, whereas 49.2% of patients showed aberrations by interphase FISH analysis. Abnormalities of *13q14*, *1q21*, *14q32* and *17p13* were detected in 27.7%, 13.8%, 16.9% and 29.2%, respectively. Patients with a *13q14* deletion or combined with *17p13* deletion frequently had a late stage of the disease, and tended to have elevated serum levels of $\beta_2$ microglobulin and lower levels of albumin. The progression-free survival and overall survival of FISH-positive patients were lower than for those without detectable abnormalities, especially in the conventional chemotherapy arm. *Conclusions:* These findings demonstrate that myeloma cells are prone to exhibiting a complex aberration and that FISH is superior to conventional cytogenetic analysis with a higher detection rate of chromosomal abnormalities. Patients with a *17p13* or *13q14* deletion, *14q32* rearrangement and *1q21* amplification were more likely to have a poor prognosis for MM.

**Key Indexing Terms:** Multiple myeloma; Cytogenetics; Fluorescence *in situ* hybridization; Clinical results. **[Am J Med Sci 2013;345(2):88–93.]**

Multiple myeloma (MM) is a malignant disorder characterized by abnormal proliferation of monoclonal immunoglobulin-producing plasma cells in the bone marrow (BM). The results from clinical trials have clearly demonstrated that patients with MM showed different clinical courses, and prognosis with overall survival (OS) ranged from months to years.[1] Studies with large samples have shown that molecular cytogenetic changes play an important role in the prognosis of MM.[1,2] Based on these findings, we tested for cytogenetic aberrations in 65 patients with MM using conventional cytogenetic analysis and the fluorescence *in situ* hybridization (FISH) technique. A retrospective study was performed on these cases to identify possible correlations with clinical features.

## PATIENTS AND METHODS

### Patients

This is a retrospective analysis of 65 patients with MM diagnosed between June 2007 and May 2010 composed of 13 relapsed cases and 52 newly diagnosed patients. The diagnostic criteria are primarily derived from the World Health Organization. We used both the International Staging System (ISS) and the Durie-Salmon (DS) staging in assessing these patients. A total of 38 male patients and 27 female patients were included, with a median age of 57 years (range, 32–81 years; IgG = 33, IgA = 18, IgD = 4, light chain = 8, nonsecretory = 2). Seven patients were in stage 1A, 15 in stage 2A, 35 in stage 3A and 8 in stage 3B by DS staging; additionally, 23 patients were in stage I, 29 in stage II and 13 in stage III by ISS staging.

Sixty-five patients with MM were eligible for inclusion in this retrospective analysis, 9 of whom either gave up treatment, returned to a local hospital for further treatment or were lost to follow-up; 2 patients with nonsecretory myeloma did not participate in an efficacy assessment. Twenty-nine of the remaining 54 patients received bortezomib-based combination chemotherapy. This treatment was composed of bortezomib plus dexamethasone or dexamethasone in combination with adriamycin, cytoxan or melphalan. The patients in this arm had a median of 3 treatment courses (1–6 courses). The other 25 patients received conventional chemotherapy including doxorubicin, vincristine and dexamethasone; melphalan plus prednisone; and melphalan, prednisone and thalidomide. The patients in this arm had a median of 4 treatment courses (2–8 courses). The efficacy assessment was according to the International Myeloma Working Group criteria.

### Conventional Cytogenetic Analysis

We obtained 5 to 10 mL of BM samples from the patients at diagnosis under sterile conditions and added RPMI 1640 with 20% fetal bovine serum and heparin. We then mixed it with colchicine amide at a concentration of 0.05 μg/mL and incubated at 37°C for 3 hours. Next, we isolated the cell suspension, followed by low permeability, and then prefixed and fixed the sample. Karyotype analysis was performed by the R-banding technique, as described in the International System for Human Cytogenetic Nomenclature (2009). Simple aberrations showed 1 to 2 incidences of chromosomal aberrations, complex aberrations showed 3 to 5 chromosomal aberrations and ultracomplex aberrations showed >6 chromosomal aberrations.

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### Interphase FISH

Five to 10 mL of BM sample, mixed with heparin, from patients with MM was obtained at diagnosis under informed consent and enriched for mononuclear cells by the Ficoll-gradient centrifugation method for further FISH detection. Probes for the regions containing *17p13*, *1q21*, *13q14*, and *14q32* and the reagents were purchased from Abbott's Vysis. (Abbott Molecular, Abbott Park, IL) BM mononuclear cells were separated by low permeability and then fixed and stored at −80°C. Slides were treated at room temperature overnight, and the hybridization area was then defined by phase-contrast microscopy. All slides were denatured, hybridized, washed and restained according to the probe reagent manufacturer's instructions.

### Signal Detection

The fluorescence microscope was purchased from Olympus (Tokyo, Japan). The *17p13*, *1q21* and *13q14* probes revealed 2 red or 2 green signals in normal interphase cells. A *1q21* probe showing >3 signals reflected a chromosomal abnormality, whereas the remaining probes with diminished signals also indicated an abnormality. In normal interphase cells, the *14q32* (*IGH*) dual-color probe appeared as 2 yellow signals resulting from a red and green signal fusion, whereas segregation referred to a disease-positive result. BM cell samples from cytogenetically normal subjects without malignant hematologic diseases were used as normal controls. We analyzed >500 interphase cells of normal BM mononuclear cells from healthy donors as controls. The cutoff levels for positive values were set at mean ± 3 standard deviations of the normal controls. The threshold values of the abnormality of *17p13*, *1q21*, *13q14* and *14q32* were 2.45%, 2.37%, 1.98% and 1.1%, respectively. Two hundred interphase cells from each patient with MM were analyzed. Positive values were indicated when the cutoff level was exceeded. Each case was examined at least twice.

### Statistical Analysis

SPSS (version 18.0) software was used for data analysis, $\chi^2$ tests were used for between-group comparisons of the discrete variables and log-rank test was used for survival analysis. A $P$ value <0.05 reflected statistical significance.

TABLE 1. The correlation between a *17p13* deletion and other detectable chromosomal abnormalities

| | ***17p13* deletion (n = 19)** | **Normal *17p13* (n = 46)** | **r** | ***P*** |
|---|---|---|---|---|
| *13q14* deletion (n = 18) | 9 | 9 | 0.283 | 0.023 |
| *14q32* rearrangement (n = 11) | 6 | 5 | 0.251 | 0.044 |
| *1q21* amplification (n = 9) | 6 | 3 | 0.330 | 0.007 |

## RESULTS

### Conventional Cytogenetic Analysis

Ten cases (15.4%) failed the analysis, and 11 (16.9%) showed abnormal cytogenetic aberrations, including 6 (9.2%) with ultracomplex aberration, 4 (6.2%) with complex aberration and 1 (1.5%) with a simple aberration. Karyotypic changes were as follows: hyperdiploid, hypodiploid, partial deletion and translocation. The numbers of patients (shown in parentheses) with these karyotypic changes are as follows—hyperdiploid: +3 (3), +5 (1), +6 (3), +9 (4), +11 (3), +12 (1), +14 (2), +15 (4), +19 (3) and +21 (1); hypodiploid: −3 (1), −6 (1), −7 (3), −9 (1), −13 (3), −16 (4), −18 (2) and −x (2); deletion: −1p (2), −6p (2), −7p (4), −1q (1), −13q (1) and −14q (2); and translocation: 2 cases with t(11;14) and t(1;14), each with t(2;11), t(1;20) and t(13;14).

### FISH Analysis

In the 65 cases examined by FISH, the overall positive rate was 49.2% (32 of 65), including 29.2% (19 of 65) with a *17p13* deletion, 27.7% (18 of 65) with a *13q14* deletion, 16.9%

TABLE 2. Fluorescence *in situ* hybridization (FISH) results and prognosis-related clinical characteristics of the 65 patients with multiple myeloma

| | **Albumin (g/L), n** | | | **$\beta_2$ microglobulin (mg/L), n** | | | | **ISS, n** | | | | **Durie-Salmon, n** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Probes (n)** | **≥35** | **<35** | ***P*** | **<2.5** | **2.5–5.5** | **≥5.5** | ***P*** | **I** | **II** | **III** | ***P*** | **1A + 2A** | **3A** | **3B** | ***P*** |
| Positive (32) | 12 | 20 | 0.034[a] | 10 | 14 | 8 | 0.574 | 8 | 16 | 8 | 0.206 | 12 | 14 | 6 | 0.184 |
| *17p13* (19) | 7 | 12 | 0.061 | 8 | 5 | 6 | 0.241 | 5 | 8 | 6 | 0.246 | 4 | 10 | 5 | 0.174 |
| *13q14* (18) | 6 | 12 | 0.037[a] | 1 | 9 | 8 | 0.009[b] | 2 | 8 | 8 | 0.018[c] | 1 | 12 | 5 | 0.027[d] |
| *1q21* (9) | 1 | 8 | 0.008[a] | 3 | 4 | 2 | 0.891 | 1 | 6 | 2 | 0.132 | 2 | 5 | 2 | 0.307 |
| *14q32* (11) | 4 | 7 | 0.164 | 4 | 5 | 2 | 1.000 | 2 | 7 | 2 | 0.269 | 3 | 6 | 2 | 0.508 |
| *17p13/13q14* (9) | 3 | 6 | 0.139 | 1 | 2 | 6 | 0.013[b] | 1 | 2 | 6 | 0.011[c] | 1 | 3 | 5 | 0.004[d] |
| *17p13/1q21* (6) | 1 | 5 | 0.068 | 2 | 3 | 1 | 1.000 | 1 | 4 | 1 | 0.355 | 2 | 3 | 1 | 0.493 |
| *17p13/14q32* (6) | 2 | 4 | 0.205 | 3 | 1 | 2 | 0.265 | 0 | 4 | 2 | 0.065[c] | 1 | 3 | 2 | 0.147 |
| >3 abnormalities (9) | 3 | 6 | 0.139 | 1 | 4 | 4 | 0.104 | 1 | 4 | 4 | 0.060 | 1 | 4 | 4 | 0.033[d] |
| Not detected (33) | 21 | 12 | — | 13 | 15 | 5 | — | 15 | 13 | 5 | — | 10 | 21 | 2 | — |

[a] There was a significant difference in the serum levels of albumin between patients with FISH-positive, *13q14* and *1q21* abnormalities and those with no detectable abnormalities ($P$ = 0.034, 0.037 and 0.008, respectively).

[b] There was a significant difference in the serum levels of $\beta_2$ microglobulin between patients with a *13q14* or *17p13*/*13q14* deletion and those with no detectable abnormalities ($P$ = 0.009 and 0.013, respectively).

[c] There was a significant difference in the ISS staging between patients with a *13q14* or *17p13*/*13q14* deletion and those with no detectable abnormalities ($P$ = 0.018 and 0.011, respectively).

[d] There was a significant difference in the Durie-Salmon staging between patients with a *13q14* or *17p13*/*13q14* deletion or patients with >3 kinds of chromosomal abnormalities and those with no detectable abnormalities ($P$ = 0.027, 0.004 and 0.033, respectively).

ISS, International Staging System.