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Clinical Utility of a Diagnostic Approach to Detect Genetic Abnormalities in Multiple Myeloma: A Single Institution Experience

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Background: The identification of genetic abnormalities in patients with multiple myeloma (MM) has gained emphasis because genetics-based risk stratification significantly affects overall survival (OS). We investigated genetic abnormalities using conventional cytogenetics and FISH and analyzed the prognostic significance of the identified additional abnormalities in MM.

Methods: In total, 267 bone marrow samples were collected from February 2006 to November 2013 from patients who were newly diagnosed as having MM in a tertiary-care hospital in Korea. The clinical and laboratory data were retrospectively obtained. Cox proportional hazard regression was used to examine the relationship between clinical/genetic factors and survival outcome, using univariate and multivariate models.

Results: Using conventional cytogenetic analysis and FISH, 45% (120/267) and 69% (183/267) patients, respectively, were identified to harbor genetic abnormalities. In the univariate analysis, the following genetic variables were identified to affect OS: abnormal karyotype ($P<0.001$), aneuploidy ($P=0.046$), -13 or del(13q) ($P=0.002$), 1q amplification ($P<0.001$), and t(4;14) ($P=0.020$). In the multivariate analysis, the presence of -13 or del(13q) was the only significant genetic factor affecting OS ($P=0.012$) with a hazard ratio (HR) of 2.131 (95% confidence interval [CI], 1.185–3.832) in addition to the clinical factor of age (>65 years) ($P=0.013$) with an HR of 2.505 (95% CI, 1.218–5.151).

Conclusions: Our findings highlight the importance of applying a comprehensive approach for detecting genetic abnormalities, which could be closely associated with the prognostic significance of MM.

Key Words: Multiple myeloma, Cytogenetics, Fluorescence *in situ* hybridization, Prognosis

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INTRODUCTION

Multiple myeloma (MM) is characterized by the clonal proliferation of plasma cells and their accumulation within the bone marrow [1]. Despite rapid advances in treatment modalities that have significantly improved patient outcomes in recent years, the prognosis of the disease is highly variable. While some patients survive for over a decade after their diagnosis, a small subset at the other end of the spectrum exhibits a highly aggressive course [2]. The presence of underlying genetic abnormalities in MM is

considered to have an important prognostic impact; thus, a number of genetic progression markers, including deletions (del) of chromosomes 13 and 17, abnormalities of chromosome 1 (1p deletion and 1q amplification), and translocations involving the immunoglobulin heavy chain (IgH), have been extensively investigated [3-7].

Conventional cytogenetic analysis is used for detecting genetic abnormalities; however, its detection rate is only 30–40% because of the relatively low proportion and proliferation rate of metaphase myeloma cells [8]. Certain cryptic aberrations such as t(4;14) and t(14;16) also hinder detection using conventional cytogenetics [2]. Conventional interphase FISH improves the detection rate; however, it is not plasma cell-specific, and the results underestimate the number of abnormal plasma cells among other cells, especially when the case is combined with a low plasma cell burden in the sample [9].

The International Myeloma Working Group recommends that a comprehensive cytogenetics/FISH evaluation should be conducted in all patients with MM at the time of diagnosis in both routine practice and clinical trials [3]. In particular, it recommends that FISH testing should be plasma cell-specific to maximize the accuracy of the results, which can be achieved by using interphase FISH either on purified plasma cells or in combination with immunofluorescent detection of cytoplasmic immunoglobulin (cIg)-FISH [3]. Although a comprehensive diagnostic approach involving plasma cell-specific FISH testing is intuitively attractive, data obtained from a large cohort of Korean patients are still insufficient.

We investigated the clinical utility of a comprehensive approach for detecting genetic abnormalities in MM at the Samsung Medical Center in Korea. In addition, we analyzed the prognostic significance of the identified additional genetic abnormalities in high-risk patients.

METHODS

1. Patients

We included 267 patients who were newly diagnosed as having MM between February 2006 and November 2013, the bone marrow samples of whom were stored at the Samsung Medical Center (a tertiary referral hospital in Korea) retrospectively. All selected patients had symptomatic plasma cell myeloma based on the WHO classification. Patients with monoclonal gammopathy of undetermined significance or smoldering myeloma or plasmacytosis with amyloidosis were excluded. Pertinent clinical and prognostic features were available for these patients, including, age, sex, performance status, stage, and levels of β_2-microglobulin, hemoglobin, lactate dehydrogenase (LDH), serum creatinine, and calcium, among others. This study was approved by the Institutional Review Board of the Samsung Medical Center (2009-11-051). Written informed consent were procured from the participating patients for genetic test.

2. Conventional cytogenetics

Conventional cytogenetic studies were performed on heparinized bone marrow samples of the patients. Each sample was cultured for 24 hours and 72 hours after lipopolysaccharide stimulation according to the protocol used routinely in clinical cancer cytogenetic laboratories [10]. After harvesting, the cells were treated with a hypotonic solution, fixed in methanol/acetic acid, and G-banded according to standard methods (3:1 ratio). At least 20 metaphase cells per patient were analyzed for karyotyping. The definitions of clones and the description of karyotypes were in accordance with the International System for Human Cytogenetic Nomenclature. Numerical chromosomal abnormalities were assigned to one of the four categories: pseudodiploid for those with 46 chromosomes and structural or numerical abnormalities, hypodiploid if they had 45 or less chromosomes, hyperdiploid if they had 47 to 74 chromosomes, and near-tetraploid if they harbored 75 or more chromosomes.

3. Probes for interphase FISH

All samples were investigated using the interphase FISH method. In our laboratory, the essential abnormalities tested included t(4;14)(p16;q32), t(14;16)(q32;q23), t(11;14)(q13;q32), deletion of 17p13 and 13q14, and 1q gain, which were analyzed simultaneously on bone marrow samples obtained from patients newly diagnosed as having MM since February 2006. The commercial probes were as follows: locus specific identifier (LSI) *IGH/FGFR3, IGH/MAF, IGH/CCND1* dual color and dual fusion translocation probes, LSI *TP53* (17p13.1)/CEP 17 dual color probe, LSI 13 (D13S319) 13q14.3 single color probe (Vysis Inc., Abbott Laboratories, Abbott Park, IL, USA) and LSI 1q21/8p21.1 dual color probe (Kreatech, Inc., Amsterdam, Netherlands). To improve laboratory diagnostic sensitivity, the cIg-FISH method was introduced in our laboratory since June 2011. Fluorescein isothiocyanate (FITC)-conjugated antibodies directed against the human κ and λ light chain were used for staining the cytoplasm of plasma cells, which allowed positive identification of plasma cells. At the same time, the *IGH/CCND1* dual color and dual fusion translocation probe (Vysis Inc., Abbott Laboratories) was replaced with the t(14;20)(q32;q12) *IGH/MAFB* probe. In

addition, the probe for 1q21/8p21 (Kreatech) was replaced by the 1q21/1p32 probe (Cytocell, Inc., Cambridge, UK).

Two hundred nuclei were scored using conventional FISH and cIg-FISH, and only the cIg-positive plasma cells were scored in cIg-FISH testing. The cut-off values for normal ranges were 0.6% for translocation probes, 3.5% for 1q21 amplification, 2.5% for 1p32 deletion, 4.3% for -13/del(13q), and 3.5% for del(17p). The normal cut-off for analysis of 200 cells was calculated using the Microsoft Excel β inverse function for obtaining the reference values for the respective probes [11]. This formula calculated a one-sided upper confidence limit for a specified percentage or proportion based on the exact computation for binomial distribution of the specimens obtained from 20 normal controls.

4. Statistical analyses and survival analyses

Statistical analyses were performed using the Chi-square test and Fisher's exact test for categorical variables and the Mann-Whitney U test or one-way analysis of variance for continuous variables. Survival analyses were performed by estimating the overall survival (OS, from the day of diagnosis to the day of death or last follow-up) in medical records. Only those patients who were diagnosed before 2013 were included in the survival analyses to obtain a sufficient follow-up period. OS was assessed using the Kaplan-Meier method. The log-rank test was used to test the differences between OS associated with chromosomal aberrations and clinical variables. Statistical significance of the factors that were associated with OS was investigated using the univariate and multivariate Cox proportional hazards regression models. Hazard ratios (HRs) and their 95% confidence intervals (CIs) were computed. The Bonferroni technique was used to evaluate the overall statistical significance of multiple comparisons. *P* values < 0.05 were considered statistically significant. All analyses were performed using SPSS version 21 (SPSS Inc., Chicago, IL, USA).

RESULTS

1. Patient characteristics

The baseline clinical and biological characteristics of 267 patients with MM are shown in Table 1. There was no difference in the baseline characteristics between patients with normal and abnormal karyotypes (see Supplemental Data Table S1).

Table 1. Clinical and biological characteristics of patients with multiple myeloma

Variable	Frequency, N (%)
Age at diagnosis, year	
Median (range)	63 (32–86)
> 65 years	112 (42)
Sex (male)	152 (57)
ECOG PS*	
0–1	187 (70)
2–4	80 (30)
Durie-Salmon staging system	
Ia/Ib	33/3 (12/1)
IIa/IIb	49/7 (18/3)
IIIa/IIIb	139/36 (52/13)
International staging system	
I/II/III/unknown	66/91/107/3 (25/34/40/1)
Paraprotein type	
Secretory	262 (98)
IgG/light chain/IgA/IgD/IgE/IgM/undetermined	140/55/51/13/1/1/1 (53/21/20/5/-/-/-)
Light chain type, kappa/lambda	134/128 (51/49)
Non-secretory	5 (2)
Hemoglobin < 100 g/L	143 (54)
Hypercalcemia (calcium ≥ 2.5 mmol/L)	60 (22)
Creatinine ≥ 176.8 μmol/L	48 (18)
β_2-microglobulin	
< 2,968 nmol/L	95 (36)
2,968–4,664 nmol/L	69 (26)
> 4,664 nmol/L	103 (39)
C-reactive protein† ≥ 47 nmol/L	100 (41)
Plasma cells in bone marrow	
< 10%	6 (2)
10–25%	59 (22)
> 25%	202 (76)
Treatment	
Thalidomide, lenalidomide or bortezomib	223 (84)
Stem cell transplantation	108 (40)

*ECOG PS score is as follows: 0 = without symptoms; 1 = mild symptoms not requiring treatment; 2 = symptoms requiring some treatment; 3 = disabling symptoms but allowing ambulation for > 50% of the day; 4 = ambulation < 50% of the day; †Data available from 244 patients.
Abbreviation: ECOG PS, Eastern Cooperative Oncology Group performance status.

2. Genetic abnormalities of the patients

Numerical and/or structural complex chromosomal abnormalities were detected in 45% (120/267) patients with MM. No mitosis was observed in 4% (10/267) patients. Among the remaining patients, 48% (128/267) harbored the normal karyotype,

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and 3% (8/267) showed Y chromosome loss as the sole abnormality. One patient with a reciprocal whole arm translocation, with break and fusion at 1p10 and 19q10, showed this abnormality in all analyzed metaphases, and the cytogenetic abnormality was considered a constitutional anomaly rather than an acquired anomaly associated with the disease.

The frequency and distribution of the identified cytogenetic abnormalities in patients with an abnormal karyotype are summarized in Table 2. Chromosomal numerical abnormalities were distributed as follows: according to number, there were 46% (55/120) hyperdiploid cases and 54% (65/120) non-hyperdiploid cases (46 hypodiploid cases, 15 pseudodiploid cases, and four near-tetraploid or more cases). Hyperdiploid karyotypes were characterized by recurrent gains of chromosomes 3, 5, 6, 7, 9, 11, 15, 18, 19, and 21. The most common trisomies of the 55 hyperdiploid karyotypes in decreasing order of occurrence were as follows: trisomies 9 (71%, 39/55), 15 (65%, 36/55), 19 (55%, 30/55), 7 (45%, 25/55), 5 (42%, 23/55), 3 (40%, 22/55), 11 (40%, 22/55), 21 (31%, 17/55), 6 (24%, 13/55), and 18 (18%, 10/55). Non-hyperdiploid karyotypes were characterized by recurrent losses of chromosomes 8, 13, and 14. The most common resulting monosomies of the 65 non-hyperdiploid karyotypes in decreasing order were as follows: monosomies 13 (57%, 37/65), 14 (25%, 16/65), and 8 (18%, 12/65) (Table 2).

Table 2. Frequencies and distribution of cytogenetic abnormalities in 120 patients with abnormal karyotype

Abnormal karyotype	Frequency, N (%)
Numerical abnormalities	
Hyperdiploid (47–74 chromosomes)	55 (46)
Non-hyperdiploid (<47 and/or >75)	65 (54)
Hypodiploid (up to 45)	46 (38)
Pseudodiploid (46)	15 (13)
Near-tetraploid or more (75 or more)	4 (3)
Structural abnormalities	
-13 or del(13q)	65 (54)
1q amplification	57 (48)
1p deletion	52 (43)
14q32 rearrangement	31 (26)
-8 or del(8p)	26 (22)
11q13 rearrangement	20 (17)
8q24 rearrangement	17 (14)
del(17p)	13 (11)
del(6q)	12 (10)
22q11 rearrangement	6 (5)

The most frequent structural abnormality was -13 or del(13q) (54%, 65/120). Structural anomalies of chromosome 1 were also frequent; 48% (57/120) patients had amplification in the long arm, and 43% (52/120) patients had deletion in the short arm. Translocations involving Ig regions were as follows: 14q32 (IgH chain locus) in 26% (31/120) patients and 22q11 (Ig lambda locus) in 5% (6/120) patients. Other structural anomalies included deletion of chromosome 6q in 10% (12/120) patients and translocations involving 8q24 in 14% (17/120) patients. Deletion involving chromosome 17p was observed in 11% (13/120) patients. Structural chromosomal anomalies involving *IGH/CCND1, IGH/FGFR3,* and chromosome 13 were significantly more common in patients with non-hyperdiploid karyotypes than in those with hyperdiploid karyotypes (see Supplemental Data Table 2).

3. Comparison of detection frequencies and distribution of genetic abnormalities observed using cytogenetics, FISH, and comprehensive cytogenetics/FISH approaches

Table 3 shows the detection frequencies obtained using the diagnostic approach. Genetic abnormalities detected by FISH alone were observed in 69% (183/267) patients, and this detection frequency was higher than that observed with conventional cytogenetics alone (45%, 120/267). The detection frequency increased up to 75% (201/267) upon combining the results obtained using conventional cytogenetics and FISH. The cumulative results of all the diagnostic approaches, including cytogenetics, FISH, and comprehensive cytogenetics/FISH showed that the detection frequencies differed significantly with respect to the identified genetic abnormalities and plasma cell burden in the bone marrow.

The comprehensive cytogenetics/FISH approach showed that the most common genetic aberration was 1q21 amplification (48%, 127/267), followed by -13 or del(13q) (39%, 103/267), 1p32 deletion (23%, 62/267), *IGH/FGFR3* rearrangement (15%, 41/267), *IGH/CCND1* rearrangement (12%, 31/267), and del (17p) (11%, 29/267). The detection frequency for the genetic abnormality was 83% (168/202) when plasma cell burden was more than 25% on the bone marrow aspirate (Table 3).

4. Prognostic significance of the identified genetic abnormalities

Two hundred and seventeen patients who were diagnosed as having symptomatic MM before 2013 were included in the survival analysis. The median follow-up duration for the patients was 24 months from the time of diagnosis. Ninety-five patients

Table 3. Differences in detection frequencies and distribution of the identified genetic abnormalities according to the diagnostic approach of cytogenetics alone, FISH alone, and a comprehensive cytogenetics/FISH approach

Category	Cytogenetics, n/N* (%)	FISH, n/N* (%)	Cytogenetics/FISH, n/N* (%)	$P^{\dagger}$
Genetic abnormalities				
amp(1q)	57/267 (21)	123/267 (46)	127/267 (48)	**<0.001**
-13 or del(13q)	65/267 (24)	97/267 (36)	103/267 (39)	**0.001**
del(1p)	52/267 (19)	6/55 (11)	62/267 (23)	0.105
IGH/FGFR3	0/267 (0)	41/267 (15)	41/267 (15)	**<0.001**
IGH/CCND1	16/267 (6)	31/156 (20)	31/267 (12)	**<0.001**
del(17p)	13/267 (5)	24/267 (9)	29/267 (11)	**0.036**
Plasma cell burden‡				
<10%	2/6 (33)	3/6 (50)	3/6 (50)	0.799
10–25%	16/59 (27)	28/59 (47)	30/59 (51)	**0.018**
>25%	102/202 (50)	152/202 (75)	168/202 (83)	**<0.001**
Total	120/267 (45)	183/267 (69)	201/267 (75)	**<0.001**

P values for comparison among cytogenetics, FISH, and cytogenetics/FISH groups were calculated using the Chi-square test. When the expected cell value was<5, Fisher's exact test was used. Significant *P* values are shown in bold.
*n/N indicates the positive number/total number of patients; †Cytogenetics versus FISH versus Cytogenetics/FISH group; ‡Proportion of plasma cells on bone marrow aspirate.
Abbreviations: amp, amplification; del, deletion.

Table 4. Univariate and multivariate Cox regression analyses of the potential factors for overall survival (OS)

Factor	Univariate model		Multivariate model	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Clinical variables				
Age (>65 years)	1.920 (1.279–2.882)	**0.002**	2.505 (1.218–5.151)	**0.013**
International staging system, stage II	2.454 (1.275–4.721)	**0.007**	0.884 (0.336–2.330)	0.804
International staging system, stage III	2.724 (1.442–5.147)	**0.002**	0.840 (0.336–2.101)	0.709
Azotemia (creatinine ≥176.8 μmol/L)	1.339 (0.829–2.165)	0.233	-	-
Lactate dehydrogenase (>480 U/L)	1.729 (1.142–2.619)	**0.010**	1.558 (0.822–2.952)	0.174
C-reactive protein (≥47 nmol/L)	1.571 (1.035–2.384)	**0.034**	1.629 (0.808–3.284)	0.172
β_2-microglobulin (>4,664 nmol/L)	1.492 (0.994–2.239)	0.053	-	-
Albumin (<35 g/L)	2.304 (1.499–3.543)	**<0.001**	1.024 (0.482–2.176)	0.950
Genetic variables				
Abnormal karyotype	2.554 (1.673–3.898)	**<0.001**	-	-
Ploidy, non-hyperdiploid group	1.759 (1.010–3.064)	**0.046**	1.900 (0.958–3.767)	0.066
-13 or del(13q)	1.901 (1.259–2.872)	**0.002**	2.131 (1.185–3.832)	**0.012**
1q21 amplification	2.161 (1.436–3.252)	**<0.001**	1.758 (0.797–3.881)	0.162
t(4;14)	1.877 (1.106–3.183)	**0.020**	1.767 (0.731–4.271)	0.206
t(11;14)	1.295 (0.732–2.290)	0.374	-	-
del(17p)	1.140 (0.592–2.198)	0.694		

In the case of multiple comparisons such as the staging system, *P* values were corrected by the Bonferroni's method. Significant *P* values are shown in bold.
Abbreviation: CI, confidence interval.

(44%, 95/217) died during the study period, and 93 patients (43%, 93/217) underwent hematopoietic stem cell transplantation. The OS rate at two years for the total number of patients was estimated at 67% with a median OS of 45 months (95% CI,

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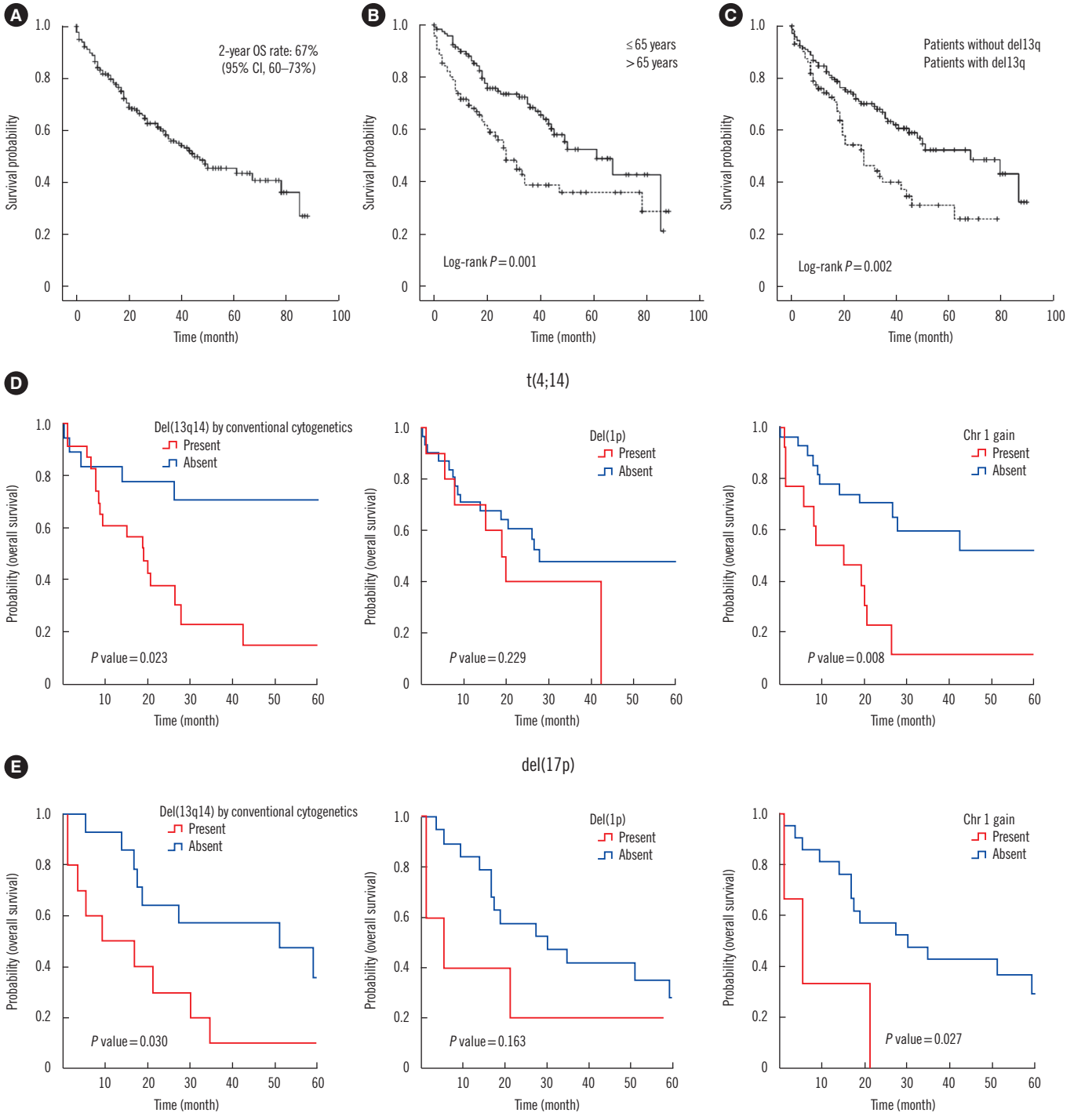

Fig. 1. Kaplan-Meier plots for the overall survival (OS) estimation and prognostic value of t(4;14) and del(17p) in multiple myeloma. (A) The OS rate at two years in 217 patients was estimated at 67%, and the median survival was 45 months (95% confidence interval [CI] 29–61 months). (B) OS according to age: ≤65 years group vs >65 years group (log-rank P=0.001). (C) OS according to the presence of del(13q) (log-rank P=0.002). (D-E) Prognostic value of t(4;14) and del(17p) in multiple myeloma.

29–61 months). Among the clinical variables, the following factors, which were evaluated using univariate Cox regression analysis, were found to affect OS: age, ISS, LDH, and levels of C-reactive protein and albumin (Table 4). Among the genetic prognostic factors obtained using cytogenetics and interphase FISH, -13 or del(13q), 1q21 amplification, and t(4;14) were signifi-

Table 5. Role of additional chromosomal changes in the prognostic value of t(4;14) and del(17p) in multiple myeloma

	t(4;14) by FISH n/N* (%)	del(17p) by FISH n/N* (%)
Conventional cytogenetics		
Hyperdiploid (47–74 chromosomes)	7/41 (17)	6/24 (25)
Non-hyperdiploid (<47 and/or >75)	20/41 (49)	9/24 (38)
Normal karyotype	10/41 (24)	8/24 (33)
-13 or del(13q)	23/41 (56)	10/24 (42)
1p deletion	10/41 (24)	5/24 (21)
1q amplification	13/41 (32)	3/24 (13)

*n/N indicates the number of positive patients out of the total number of examined patients.

cant. Abnormal karyotype and the presence of chromosomal aneuploidy also affected OS. In the multivariate analysis using these clinical and genetic factors, only age (>65 years) and presence of -13 or del(13q) were significant factors for OS. Kaplan-Meier plots for OS, total series, and OS according to significant prognostic factors, such as age and -13 or del(13q), are illustrated (Fig. 1A-C).

5. Role of additional chromosomal changes in the prognostic value of t(4;14) and del(17p) in MM

We analyzed the role of additional chromosomal changes in the prognostic value of t(4;14) and del(17p) in MM (Table 5 & Fig. 1D, E). A certain degree of heterogeneity existed in the survival of high-risk patients. Additional chromosomal changes modulated the outcome of patients with high-risk MM. In patients with t(4;14), -13 or del(13q) and chromosome 1q gain negatively impacted OS. In patients with del(17p), del(13q) and chromosome 1q gain also negatively impacted OS (Fig. 1D, E).

DISCUSSION

Genetic abnormalities in patients with MM are typically complex, involving several chromosomal alterations in terms of both number and structure. Recently, the identification of genetic abnormalities in patients with MM has attracted attention because genetics-based risk stratification significantly affects OS [3, 4]. Identifying high-risk diseases at an early stage might potentially influence the strategies for patient monitoring and management.

In the present study, genetic abnormalities were identified in 45% (120/267) of the patients, using conventional cytogenetics, and this value increased to 75% (201/267) after including the results that were determined using interphase FISH. Genetic abnormalities detected by FISH alone were observed in 69% (183/267) of the patients. This detection frequency was higher than that observed using conventional cytogenetics alone (45%, 120/267), suggesting that FISH can be a far more sensitive method for detecting genetic changes in MM. The detection frequencies obtained with conventional cytogenetics are similar to that reported (21–42%) in previous Korean studies [12-15]. Genetic abnormalities detected by FISH range from 38% to 86% [12-15], which might depend on the number of probes tested in these studies. The high proportion of abnormal plasma cells detected by cIg-FISH eliminated the ambiguities associated with the results of conventional FISH (see Supplemental Data Fig. S1), particularly in cases with low proportion of abnormal plasma cells that were close to the normal cut-off value [9]. The improved analytical sensitivity and specificity of cIg-FISH are important because of reliable risk stratification with higher certainty.

In the current study, -13 or del(13q) was observed in 65 patients (54% patients with abnormal karyotype), and it was the most frequently observed abnormality. The genetic abnormality of -13 or del(13q) is generally associated with poor prognosis, and we showed that these abnormalities significantly affect survival (Table 4). Chromosome 1 abnormality was common. Previous studies have reported that the frequency of the 1q amplification ranges from 17 to 45% [12-14]. In the present study, 57 patients (48% patients with an abnormal karyotype) harbored the 1q amplification, as identified using conventional cytogenetics, which is similar to the frequency reported previously. A study shows that 1q amplification is related to poor clinical outcomes, such as a lower hemoglobin level and a higher plasma cell burden [12]. We also confirmed that the 1q amplification is a potential factor affecting survival, although it was not significant according to the multivariate Cox regression analysis. IgH rearrangement was the third most prevalent change found in 31 patients (26% patients with an abnormal karyotype). Translocation involving the IgH chain locus is the most common abnormality seen in MM, and the presence of t(4;14), t(4;16), and t(14;20) typically signifies a poor clinical outcome [16].

A large number of potential prognostic factors have been identified over the years. Several staging systems have been introduced to predict the prognosis of the disease, including the ISS and Durie-Salmon staging system. The Durie-Salmon staging system was the first most commonly used staging system [17]. Since the mid-1970s, the Durie-Salmon staging system has been widely used for MM; this system uses several variables, including levels of hemoglobin, calcium, and monoclonal protein, and the number of bone lesions and creatinine [17]. The ISS is a

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new staging system, which separates patients with MM into three groups based on β_2-microglobulin and albumin levels, which are two important prognostic factors for patients with MM [18]. In our study, the ISS, but not the Durie-Salmon staging system, was a potential factor affecting survival. Recently, cytogenetic risk stratification at diagnosis and relapse has increasingly become an integral part of the standard care that predicts OS and progression-free survival independent of the ISS. Thus, a combination of modified ISS with FISH data has been introduced in risk assessment [19, 20].

In summary, our findings support the claim that a comprehensive cytogenetics/FISH approach could be an effective tool for the detection of genetic abnormalities, which have increasingly become an integral part of risk stratification and disease management that predict OS. Additional chromosomal changes modulated the outcome of patients with high-risk MM. In patients with t(4;14), del(13q) and chromosome 1q gain negatively impacted OS. Our findings imply the importance of applying a comprehensive approach to detect genetic abnormalities, which might be closely associated with the prognosis of MM.

Authors' Disclosures of Potential Conflicts of Interest

The authors declare that no conflicts of interest exist.

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Supplemental Data Table S1. Comparison between patients with normal and abnormal karyotypes

Variable	Normal karyotype Frequency, n/N* (%)	Abnormal karyotype Frequency, n/N* (%)	*P*
Age (>65 years old)	53/128 (41)	49/120 (41)	0.927
Sex (male)	75/128 (59)	62/120 (52)	0.273
ECOG PS† (0–1)	90/128 (70)	83/120 (69)	0.844
ISS I	36/128 (28)	23/119 (19)	0.098
ISS II	40/128 (31)	47/119 (39)	0.192
ISS III	50/128 (39)	49/119 (41)	0.776
Unknown	2/128 (2)	1/120 (1)	1.000
Azotemia (creatinine ≥176.8 μmol/L)	21/127 (17)	22/120 (18)	0.710
β_2-microglobulin (>4,664 nmol/L)	48/126 (38)	47/119 (39)	0.822
Albumin (<35 g/L)	55/127 (43)	53/120 (44)	0.892

*n/N indicates the number of positive patients out of the total number of examined patients; †ECOG PS score: 0, without symptoms; 1, mild symptoms not requiring treatment.
Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; ISS, international staging system.

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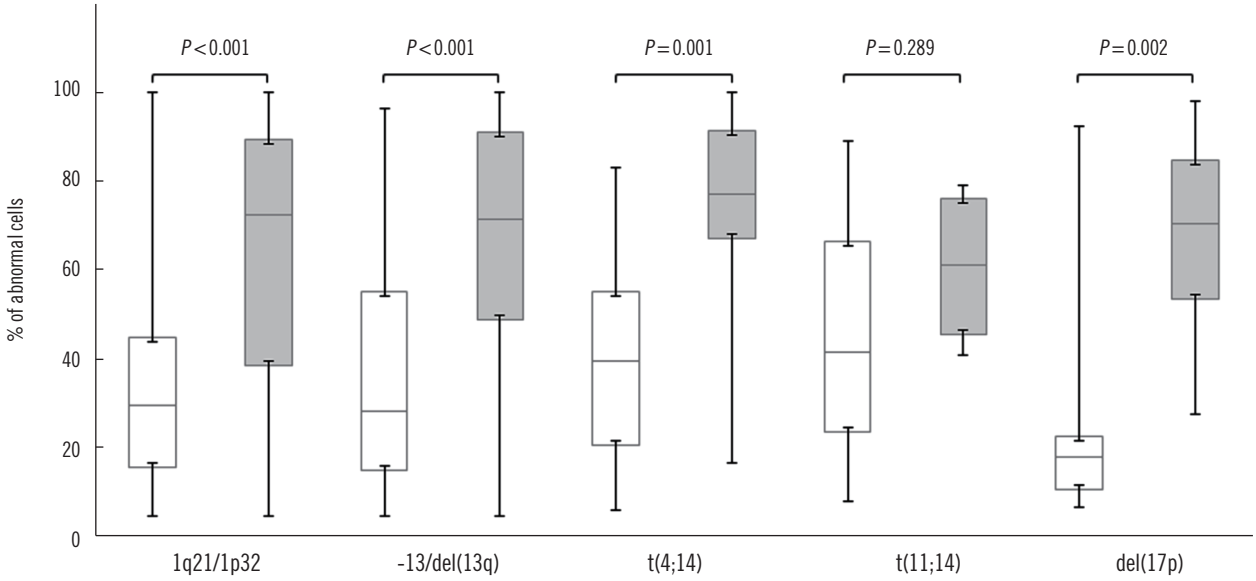
Supplemental Data Table S2. Type and distribution of FISH data in the total series and according to the cytogenetic subgroup

Structural abnormalities by FISH	Total series n/N* (%)	Normal karyotype n/N* (%)	Hyperdiploid group n/N* (%)	Non-hyperdiploid group, n/N* (%)	$P^{\dagger}$	$P^{\ddagger}$
IGH rearrangement						
IGH/CCND1[§]	31/156 (20)	15/78 (19)	2/28 (7)	13/35 (37)	**0.012**	**0.005**
IGH/FGFR3	41/267 (15)	10/128 (8)	7/55 (13)	20/65 (31)	**<0.001**	**0.018**
IGH/MAF	1/267 (0)	1/128 (1)	0/55 (0)	0/65 (0)	NA	NA
IGH/MAFB[§]	1/115 (1)	0/50 (0)	0/27 (0)	1/34 (3)	NA	NA
-13 or del(13q)	97/267 (36)	31/128 (24)	22/55 (40)	42/65 (65)	**<0.001**	**0.007**
amp(1q)	123/267 (46)	44/128 (34)	32/55 (58)	43/65 (66)	**<0.001**	0.369
del(17p)	24/267 (9)	8/128 (6)	6/55 (11)	9/65 (14)	0.204	0.628
del(1p)[‖]	6/55 (11)	2/19 (11)	2/14 (14)	2/22 (9)	0.886	0.634

*n/N indicates the positive number/total number of patients; [†]Normal karyotype versus hyperdiploid group versus non-hyperdiploid group; [‡]Hyperdiploid group versus non-hyperdiploid group; [§]The *IGH/CCND1* probe has been replaced by the *IGH/MAFB* probe since June 2011; [‖]Probe for 1p deletion has been used since June 2011.
Abbreviations: FISH, fluorescence *in situ* hybridization; NA, not applicable; IGH, immunoglobulin heavy locus.



Supplemental Data Fig. S1. Box-Whisker plot for distribution of abnormal cells (%) between conventional fluorescence *in situ* hybridization (FISH) and plasma cell-specific cytoplasmic immunoglobulin FISH (cIg-FISH) according to the tested probe type. Values for conventional FISH are represented by white boxes, whereas those for cIg-FISH are represented by gray boxes.

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