Development and validation of prognostic implications of chromosome abnormalities algorithm for newly diagnosed multiple myeloma

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Abstract

Fluorescence in situ hybridization (FISH) evaluation is essential for initial risk stratification in multiple myeloma (MM). The presence of specific cytogenetic abnormalities (CA) confers a heterogeneity impact on prognosis. However, the cutoff values among different centers are not uniform. Therefore, we conduct this study to better predict the prognosis of newly diagnosed MM patients based on FISH results. The Kaps method was used to calculate the chromosomal abnormal cutoff values. A total of 533 participants were included in the study. The best cutoff value of overall survival were as follows: 17p− 20.1%, 13q− 85%, 1q21+ 39%, t(11;14) 55.5%, t(14;16) 87%, and t(4;14) 53.5%. The survival analysis showed that 17p− and 1q21+ were the independent factors affecting both OS and progress free survival (PFS) among CA. The analysis based on the cutoff value obtained by Kaps suggested that 13q−, t(14;16), 17p−, and 1q21+ were independent factors affecting OS among CA; t(14;16), 17p−, and 1q21+ were independent factors affecting PFS among CA. The prognostic model was constructed by the Kaps method with the Harrell concordance index (c-index) at 0.719 (95% CI, 0.683–0.756; corrected 0.707), which was higher than that calculated by the European Myeloma Network criteria (0.714; 95% CI, 0.678–0.751; corrected 0.696). In conclusion, chromosomal abnormalities in different proportions and combinations can affect the prognosis of MM patients. Therefore, effective criteria should be formulated to evaluate the prognosis of MM patients better.

Keywords: Multiple myeloma, Fluorescent in situ hybridization, Chromosome aberrations, Cytogenetic analysis, Prognosis

1. INTRODUCTION

As a malignant disease of plasma cells, multiple myeloma (MM) had been reported that there were significant differences in the clinical manifestations, prognosis, and response to treatment in MM patients with different cytogenetic abnormalities (CA).[1] The process of MM formation is the process of CA changing accompanying the clinical characteristics of MM patients changing.[2] Recently, fluorescence in situ hybridization (FISH) became a standard method for the characterization and quantification of CA in MM patients. However, the detecting methods and the definition of cutoff value were still controversial. The European Myeloma Network (EMN) criteria recommended that the cutoff value should be 20% for abnormal chromosome numbers, the cutoff level should be 10% for IgH translocations and other translocations.[3] The cutoff values that scholars recommended for 17p−, 1q21+, and 13q− was 60%, 30%, and 74%, respectively in the IFM 99 test,[4] and for 1q21+, 13q− was 20% and 10%, respectively at the Mayo Center.[5]

How to assess the risk of the MM patients by FISH results is still controversial, the clinical significance of the proportion of different chromosomal abnormalities detected by FISH is also unclear. Therefore, we conduct this study to investigate the prognostic implications of chromosome abnormalities for newly diagnosed MM patients and provide a more accurate prediction of the overall survival (OS) of MM patients in the real world.

2. RESULTS

2.1. Characteristics of patients

A total of 533 patients who were newly diagnosed as MM were included in this study. Among these 533 patients, there were

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WF and JD contributed equally to this work.

TL, WQ, and JL collected and analyzed the data, and wrote the first draft, and approved the final version of the manuscript. All authors performed patient management and approved the final version of the manuscript. JL, LL, HH, and HJ performed patients' follow-up, participated in the final data analysis and approval of the final version of the manuscript. WF and JH designed the study, performed patient management, and approved the final version of the manuscript; and JD designed the study, performed patient management, analyzed the data, wrote the first draft, and approved the final version of the manuscript.

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The datasets analyzed during the present study are available from the corresponding authors on reasonable request.

This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the Second Military Medical University (Ethical number: 2016SL019). All participants had signed the informed consent.

The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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325 males (60.98%) and 208 females (39.02%). The median age of the patients was 61 years old (range, 23–87 years old). All patients received at least 1 novel agent, 476 patients (89.31%) contained bortezomib, 103 patients (19.32%) contained lenalidomide, 282 patients (52.91%) contained thalidomide, followed by stem cell transplants if possible. The median follow-up time was 35.79 (0.21–88.88) months. A total of 324 patients (60.8%) survived at the end of the follow-up. All features of the patients are detailed in Table 1.

2.2. The effect of single CA on survival

According to the EMN criteria, we investigated the single CA aberration on impaction of prognosis at different clone sizes. The results presented that patients harbored 17p−, 13q−, and 1q21+ showed shorter OS and patients harbored 17p− and 1q21+ have shorter PFS based on the EMN criteria. Then, we used Kaps to calculate the best cutoff value of OS and the results were as follows: 17p− 20.1%, 13q− 85%, 1q21+ 39%, t(11;14) 55.5%, t(14;16) 87%, and t(4;14) 53.5%. Based on the cutoff value calculated by Kaps, we also found that patients harbored 17p−, 13q−, 1q21+, t(4;14), t(11;14), and t(14;16) showed shorter OS and patients harbored 17p−, 13q−, 1q21+, t(11;14), and t (14;16) have shorter PFS (Fig. 1). The details were listed in Table 2.

To identify which of the single CA aberration was really affecting the prognosis of patients, we further performed a multivariate analysis of all CA and other possible survival-related parameters by Cox stepwise regression. Firstly, we analyzed the prognostic factors of PFS or OS according to the EMN criteria. The statistically independent predictors of PFS were 1q21+, 17p−, ISS stage, LDH, M-spike, gender, and transplantation schemes. The statistically independent predictors of OS were 1q21+, 17p−, age, ISS stage, LDH, DS stage, and M-spike. After that, we analyzed the prognostic factors of PFS or OS according to the cutoff value calculated by Kaps and the results showed that the statistically independent predictors of PFS were 1q21+, 17p−, t(14;16), ISS stage, LDH, isotype, and transplantation schemes. The statistically independent predictors of OS were 1q21+, 17p−, t(14;16), 13q−, age, ISS stage, LDH, and M-spike. The details were listed in Tables 3 and 4.

Table 1

Clinical and laboratory characteristics of patients

Characteristics	(N = 533)
Gender	
Male	325 (60.98%)
Female	208 (39.02%)
Age at diagnosis [median (range)]	61 (23–87)
Isotype	
IgA	126 (23.64%)
IgD	46 (8.63%)
IgG	250 (46.90%)
sFLC only	98 (18.39%)
Non-secretory and other	13 (2.44%)
DS stage	
IA	14 (2.63%)
IB	1 (0.19%)
IIA	26 (4.88%)
IIB	2 (0.38%)
IIIA	373 (69.98%)
IIIB	117 (21.95%)
ISS stage	
I	172 (32.27%)
II	162 (30.39%)
III	199 (37.34%)
LDH [U/L, median (range)]	162 (66–819)
PLT[$\times 10^9$/L, median (range)]	166 (13–501)
HB [g/L, median (range)]	91 (38–159)
Cr [μmol/L, median (range)]	80 (27–1388)
ALB [g/L, median (range)]	36 (13–55)
β2M [mg/L, median (range))]	3.85 (0.63–106)
Ca [mmol/L, median (range)]	2.4 (1.37–3.9)
Clonal BM plasma cells (%)	31 (0–100)
Treatment	
Contain bortezomib	476 (89.31%)
Contain lenalidomide	103 (19.32%)
Contain thalidomide	282 (52.91%)
Transplantation schemes	
Non	432 (81.20%)
Autologous stem cell transplantation	89 (16.73%)
Allogeneic stem cell transplantation	11 (2.07%)
FISH	
17p− ≥20%	68 (12.76%)
13q− ≥20%	243 (45.59%)
Iq21+ ≥20%	294 (55.16%)
IgH translocation ≥10%	352 (66.04%)
t(11;14) ≥10%	71 (13.32%)
t(4;14) ≥10%	93 (17.45%)
t(14;16) ≥10%	10 (1.88%)

β2M = β2-microglobulin, ALB = albumin, BM = bone marrow, Ca = serum calcium, Cr = creatinine, DS stage = Durie–Salmon stage, FISH = fluorescence in situ hybridization evaluation, HB = hemoglobin, ISS stage = International Staging System stage, LDH = lactate dehydrogenase, PLT = platelet, sFLC = serum free light chain.

2.3. Prediction model and validation and calibration

To further verify that the criteria calculated by Kaps can predict survival more accurately, we constructed 2 prognostic models for both criteria according to the Cox multivariate analysis results of OS. We used nomograms to visualize the prediction models first (Fig. 2), then used the calibration curve and Harrell concordance index (c-index) to evaluate the performance of the prediction models. The results showed that the c-index for the nomogram established by the Kaps method to predict OS was 0.719; 95% CI, 0.683 to 0.756; corrected 0.707, and the c-index for the nomogram that calculated by the EMN criteria was 0.714; 95% CI, 0.678 to 0.751; corrected 0.696. The calibration curve of the 2 prognostic models was shown in Figure 3.

2.4. The impact of adverse CA number on prognosis

Finally, we analyzed the influence of the number of adverse CA on prognosis. According to the result of multivariate analysis which was calculated by the EMN criteria, we found that there were 2 adverse lesions: 17p− and 1q21+. Then, patients were divided into 3 groups: no abnormalities (204 patients, 38.27%), 1 abnormality (296 patients, 55.53%), and 2 abnormalities (33 patients, 6.19%). The results of the univariate Cox regression analysis showed that the OS [HR, 1.984 (95% CI, 1.452–2.709) $P < 0.001$] and PFS [HR, 1.740 (95% CI, 1.357–2.232), $P < .001$] of 1 abnormality group and the OS [HR, 2.920 (95% CI, 1.715–4.971), $P < .001$] and PFS [HR, 3.046 (95%CI, 1.948–4.762), $P < .001$] of 2 abnormalities group were shorter than that of the no abnormalities group. The survival curves were shown in Figure 4A and B. Multivariate analysis showed that there were 2 independent prognostic factors associated with PFS in the 3 groups: 1 abnormality group [HR, 1.706 (95% CI, 1.313–2.217), $P < .001$] and 2 abnormalities group [HR, 2.811 (95% CI, 1.762–4.485)], $P < .001$]. There were also 2 independent

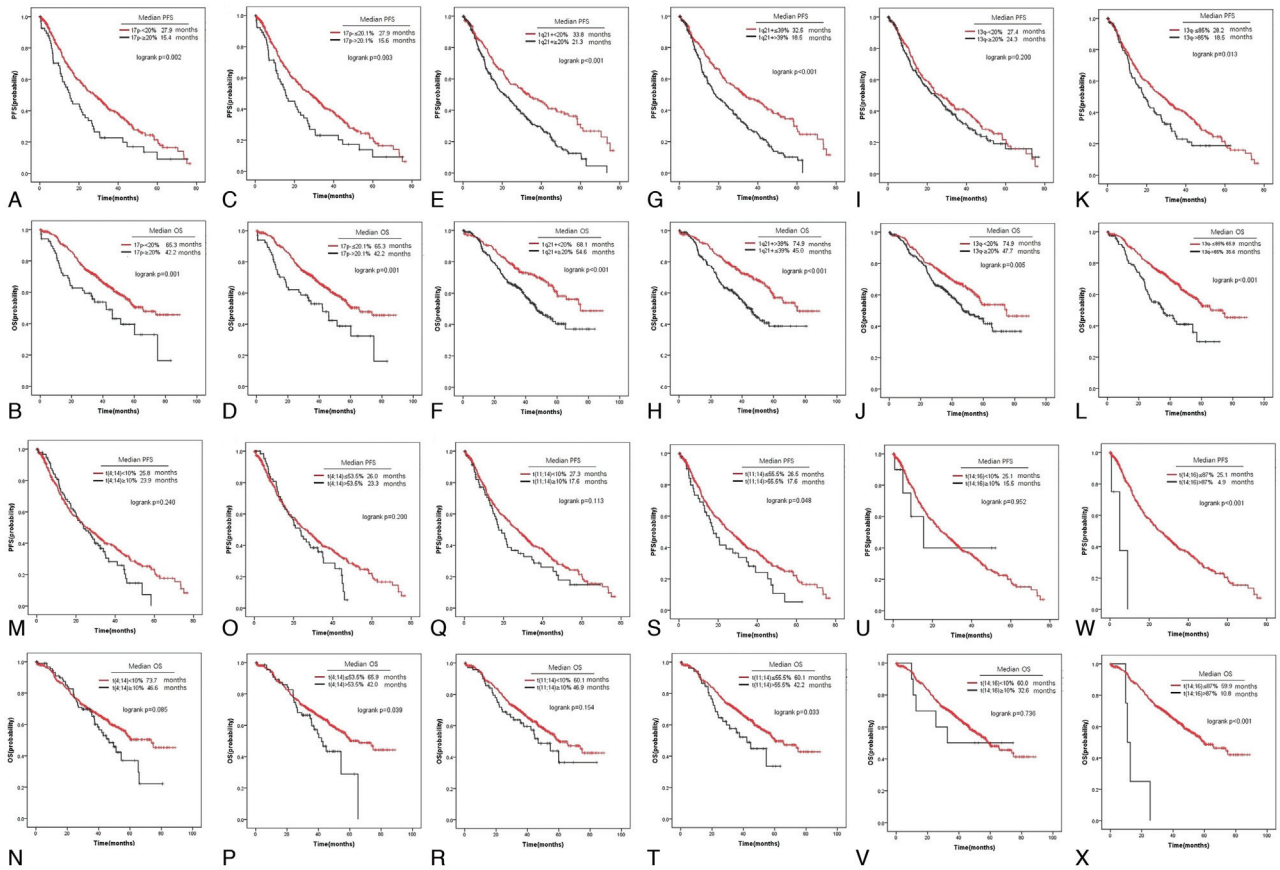


Figure 1. Survival analysis. Survival analysis for patients with 17p− ≥20% vs 17p− <20%(A-B), 17p− >20.1% vs 17p− ≤20.1%(C-D), 13q− ≥20% vs 13q− <20%(E-F), 13q− >85% vs 13q− ≤85%(G-H), 1q21+ ≥20% vs 1q21+ <20%(I-J), 1q21+ >39% vs 1q21+ ≤39%(K-L), t(4;14) ≥10% vs t(4;14) <10%(M-N), t(4;14) >53.5% vs t(4;14) ≤53.5%(O-P), t(11;14) ≥10% vs t(11;14) <10%(Q-R), t(11;14) >55.5% vs t(11;14) ≤55.5%(S-T), t(14;16) ≥10% vs t(14;16) <10%(U-V), t(14;16) >87% vs t(14;16) ≤87%(W-X).

prognostic factors associated with OS: 1 abnormality group [HR, 1.887 (95% CI, 1.355–2.630) $P < .001$] and 2 abnormalities group [HR, 2.780 (95% CI, 1.566–4.934), $P < .001$].

The results of multivariate analysis showed there were 4 adverse lesions based on the criteria calculated by the Kaps method: 17p−, 1q21+, 13q−, and t(14;16). Then, patients were divided into 4 groups: no abnormalities (208 patients, 39.02%), 1 abnormality (228 patients, 42.78%), 2 abnormalities (86 patients, 16.14%), and more than 2 abnormalities group (11 patients, 2.06%). The results of the univariate Cox regression analysis showed that the OS [HR, 1.595 (95% CI, 1.147–2.219) $P = .006$] and PFS [HR,1.426 (95% CI, 1.103–1.844), $P = .007$] of 1 abnormality group, the OS [HR, 3.152 (95% CI, 2.161–4.597) $P < .001$] and PFS [HR, 2.385 (95% CI, 1.722–3.305), $P < .001$] of 2 abnormalities group, and the OS [HR, 12.755 (95% CI, 6.426–25.318), $P < .001$] and PFS [HR, 7.032 (95% CI, 3.720–13.292), $P < .001$] of more than 2 abnormalities group were shorter than that of the no abnormalities group. The survival curves were shown in Figure 4C and D. Besides, multivariate analysis showed that there were 3 independent prognostic factors associated with PFS: 1 abnormality group [HR, 1.347 (95% CI, 1.029–1.762), $P = .030$], 2 abnormalities group [HR, 2.281 (95% CI, 1.627–3.199), $P < .001$], and more than 2 abnormalities group [HR, 7.766 (95% CI, 3.849–15.667), $P < .001$]. There were also 3 independent prognostic factors associated with OS: 1 abnormality group [HR, 1.501 (95%CI, 1.059–2.128), $P = .023$], 2 abnormalities group [HR, 2.773 (95% CI, 1.864–4.127), $P < .001$], and more than 2 abnormalities group [HR, 17.310 (95% CI, 7.972–37.583), $P < .001$].

3. DISCUSSION

The outcomes of this study presented that CA can affect the prognosis of MM patients with different proportions and combinations. According to the clinical data of this study, the cutoff values of CA calculated by Kaps may have clinical significance were as follows: 17p− 20.1%, 13q− 85%, 1q21+ 39%, and t(14;16) 87%. If there were more types of adverse CA, the prognosis of MM patients might be worse.

MM has the characteristics of wide heterogeneity in clinical manifestations and prognosis. The intrinsic mechanism may be related to the structural and quantitative changes of many chromosomes and the oncogene and tumor suppressor gene mutation in MM.[7] The diversity of the proportion of cells with specific mutations, the loss of gene function caused by some mutations, the differences in mutation sites between different patients, and the constant evolution of myeloma cells make targeted treatment very difficult.[8] Therefore, it was very important to predict the prognosis of different MM patients based on their clinical characteristics. At present, the prognostic

Table 2

The results of the univariate Cox regression analysis

	EMN criteria				The cutoff value calculated by Kaps			
	PFS		OS		PFS		OS	
Characteristics	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
1q21+	1.672 (1.326–2.109)	<.001	1.729 (1.301–2.299)	<.001	1.775 (1.416–2.224)	<.001	1.910 (1.448–2.520)	<.001
17p−	1.606 (1.186–2.175)	.002	1.803 (1.258–2.582)	.001	1.574 (1.159–2.136)	.004	1.850 (1.291–2.650)	.001
13q−	1.156 (0.926–1.445)	.201	1.478 (1.126–1.941)	.005	1.395 (1.071–1.817)	.014	2.038 (1.504–2.762)	<.001
t(4;14)	1.182 (0.894–1.561)	.241	1.343 (0.959–1.881)	.086	1.231 (0.895–1.693)	.201	1.486 (1.017–2.172)	.041
t(11;14)	1.285 (0.942–1.754)	.114	1.314 (0.901–1.916)	.156	1.412 (1.001–1.991)	.049	1.562 (1.033–2.359)	.034
t(14;16)	1.031 (0.384–2.768)	.952	1.165 (0.479- 2.833)	.736	8.321 (2.628–26.349)	<.001	8.186 (3.005–22.299)	<.001

EMN criteria = the European Myeloma Network criteria, OS = overall survival, PFS = progress free survival.

evaluation system for MM included the DS stage, ISS stage, R-ISS stage, mSMART stage, etc.[9–12] Among them, the DS and ISS stage only had the conventional prognostic factors but not the cytogenetic indicators because these 2 stages were submitted earlier. With the deepening of the understanding of the disease, 17p−, 1q21+, t(4;14), t(14;16), etc were found to be prognostic factors,[13–15] then R-ISS staging, mSMART staging were submitted, added with cytogenetic factors and to predict the prognosis of MM patients more accurately. FISH was currently the standard method for the characterization and quantification of CA in MM patients. Furthermore, FISH has become an indispensable tool in the course of diagnosis and subsequent personalized treatment.

However, it was still a controversial issue that what was the optimal percentage of abnormal cytology detected by FISH can be considered positive, which may lead to poor prognosis and further treatment.[1] The cutoff values of different chromosomes in different centers were inconsistent. For example, the cutoff value of 17p− varied from 10% to 60%.[16–20] Some scholars have suggested giving an optimal cutoff value, but a more accurate cutoff value is needed in clinical practice to sensitively and efficiently judge the prognosis. To further explore the effect of different percentages and combinations of CA detected by FISH on the prognosis of MM patients, we did not artificially distinguish each chromosomal abnormality like the study by Gang An et al,[21] which may not be able to make the best distinction between continuous variables. We calculated the optimal cutoff value of different chromosomal abnormalities by Kaps. This method was also used in the Revised International Staging System for Multiple Myeloma.[11] It could analyze based on the actual patient's OS and provided a minimum partition by log-rank test and find a set of optimal cutoff points without establishing the number and scope of groups in advance,[6] which can be called a more accurate prediction in the real world.

Table 3

Multivariate analysis results according to the EMN criteria

	PFS			OS	
Characteristics	HR (95% CI)	*P* value	Characteristics	HR (95% CI)	*P* value
1q21+	1.699 (1.328–2.173)	<.001	1q21+	1.713 (1.258–2.331)	<.001
17p−	1.670 (1.214–2.297)	.002	17p−	1.794 (1.219–2.640)	.003
ISS stage			Age	1.019 (1.003–1.035)	.022
I	Reference		ISS stage		
II	1.073 (0.788–1.461)	.654	I	Reference	
III	1.831 (1.372–2.442)	<.001	II	1.236 (0.808–1.890)	.328
LDH	1.002 (1.001–1.002)	<.001	III	2.410 (1.639–3.544)	<.001
Isotype			LDH	1.002 (1.001–1.003)	<.001
IgA	Reference		DS stage		
IgG	1.172 (0.858–1.601)	.318	I	0.217 (0.030–1.561)	.129
IgD	1.952 (1.291–2.951)	.002	II	0.350 (0.128–0.957)	.041
sFLC only	0.990 (0.676–1.450)	.959	III	Reference	
Non-secretory and other	1.658 (0.748–3.676)	.213	Isotype		
Gender			IgA	Reference	
Male	Reference		IgG	1.403 (0.947–2.078)	.091
Female	0.759 (0.595–0.969)	.027	IgD	2.197 (1.313–3.675)	.002
Transplantation schemes			sFLC only	0.718 (0.429–1.203)	.209
Non	Reference		Non-secretory and other	0.888 (0.312–2.524)	.824
ASCT	0.667 (0.487–0.914)	.012			
alloSCT	0.776 (0.342–1.760)	.545			

alloSCT = allogeneic stem cell transplantation, ASCT = autologous hematopoietic stem cell transplantation, DS stage = Durie–Salmon stage, EMN = the European Myeloma Network, ISS stage = International Staging System stage, LDH = lactate dehydrogenase, OS = overall survival, PFS = progress free survival, sFLC = serum free light chain.

Table 4

Multivariate analysis results according to the cutoff value calculated by Kaps

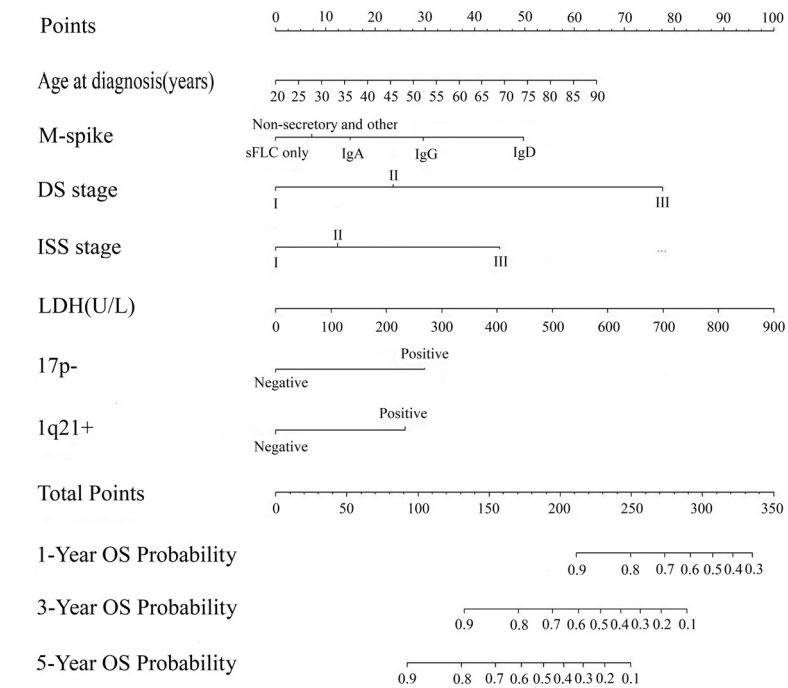
	PFS			OS	
Characteristics	**HR (95% CI)**	***P* value**	**Characteristics**	**HR (95% CI)**	***P* value**
1q21+	1.759 (1.386–2.231)	<.001	1q21+	1.719 (1.272–2.323)	<.001
17p−	1.647 (1.196–2.269)	.002	17p-	1.891 (1.286–2.779)	.001
t (14; 16)	5.107 (1.224–21.315)	.003	t (14; 16)	3.948 (1.216–12.821)	.022
ISS stage			13q−	1.899 (1.357–2.656)	<.001
I	Reference		Age	1.020 (1.004–1.036)	.016
II	1.077 (0.792–1.465)	.638	ISS stage		
III	1.881 (1.411–2.508)	<.001	I	Reference	
LDH	1.001 (1.000–1.002)	.002	II	1.322 (0.864–2.020)	.198
Isotype			III	2.743 (1.872–4.018)	<.001
IgA	Reference		LDH	1.002 (1.001–1.003)	<.001
IgG	1.142 (0.837–1.557)	.403	Isotype		
IgD	2.044 (1.352–3.089)	<.001	IgA	Reference	
sFLC only	0.977 (0.668–1.429)	.903	IgG	1.249 (0.847–1.840)	.262
Non-secretory and other	1.548 (0.698–3.434)	.283	IgD	2.245 (1.341–3.760)	.002
Transplantation schemes			sFLC only	0.682 (0.408–1.141)	.145
Non	Reference		Non-secretory and other	1.022 (0.359–2.912)	.967
ASCT	0.656 (0.479–0.900)	.009			
alloSCT	0.732 (0.323–1.656)	.454			

alloSCT = allogeneic stem cell transplantation, ASCT = autologous hematopoietic stem cell transplantation, ISS stage = International Staging System stage, LDH = lactate dehydrogenase, OS = overall survival, PFS = progress free survival, sFLC = serum free light chain.

In the multivariate analysis, t(11;14) and t(4;14) were not independent prognostic factors of MM patients ($P > .05$) no matter the analysis was based on the EMN criteria or based on the cutoff value calculated by Kaps. However, t(4;14) was considered as a prognostic factor in previous studies,[22,23] while bortezomib seemed to improve its adverse effects.[24–26] In this study, among the patients with t(4;14) ≥10%, those who underwent the chemotherapy regimens that contained bortezomib accounted for 89.36%. It seemed that the survival improvement of patients with t(4;14) may be due to the higher ratio of bortezomib usage. As for another adverse prognostic factor, t(14;16) was not the prognostic factor of MM patients in both univariate and multivariate analysis according to the EMN criteria.[27] However, it became the prognostic factor in both univariate and multivariate analysis according to the cutoff value calculated by Kaps. This indicated that although t(14;16) has a prognostic disadvantage, the cutoff value of t(14;16) will affect the result of the analysis, and resetting the cutoff value seemed necessary. The t(11;14) causes upregulation of cyclin D1 and has been considered as a favorable or innocuous factor for prognosis, which is consistent with the findings in this study.[28] Among all CA of MM, 17p− may be the most important prognostic factor, accounting for 10% to 20% of newly diagnosed MM patients, and mostly present in patients with IgH translocation.[29] The current primary treatment regimen did not improve the prognosis of patients with 17p−.[30] In this study, we calculated the 17p− using the optimal cutoff value of 20.1% by Kaps and the results showed that 17p− were an unfavorable prognostic factor whether based on univariate analysis or multivariate analysis. Because the EMN recommended the cutoff values be relatively conservative,[3] it indicated 17p− has a great impact on the prognosis of patients, thus special attention in clinical treatment is needed.

Many previous studies have shown that 1q21+ can lead to poor prognosis in patients with MM,[1,4,31,32] which was consistent with our findings. In this study, the criteria according to the EMN and the criteria calculated by Kaps both found that 1q21+ was an independent prognostic factor in both univariate and multivariate analysis. Compared with the EMN criteria, univariate analysis showed that HR values of PFS and OS were higher when 39% were taken as cutoff values. Therefore, if 39% were taken as cutoff value, it can distinguish the effect of 1q21+ on prognosis more clearly. Furthermore, with the increase of 1q21+ percentage, the prognosis of patients gradually deteriorates.

Recently, Walker et al found that the copy number of CKS1B (1q21) was related to the prognosis of MM patients, and the prognosis of patients with amplification (≥4 copies) of CKS1B (1q21) on the background of International Staging System III was extremely poor.[33] This may indicate that with the increase of 1q21+ percentage, the copy number of CKS1B also increases. Therefore, the FISH detection in subsequent studies should be clear about the changes in the copy number of CKS1B. The effect of 13q− on prognosis was highly controversial. Some studies suggested that 13q− may lead to poor prognosis,[34] while other studies suggested that 13q− alone does not worsen the prognosis of MM patients, but when 13q− was associated with 17p− and t(4;14) leads to poor prognosis.[16,35] In this study, we first performed a survival analysis of patients according to the EMN criteria and found that although there was a statistical difference between OS in 13q− positive and 13q− negative patients in the univariate analysis, 13q− was not an independent prognostic factor in the multivariate analysis. Then according to the 85% cutoff value calculated by Kaps, it was found that 13q− was an independent prognostic factor no matter based on univariate analysis or multivariate analysis. Because the stepwise regression method was used for multivariate analysis in this study, it can be ruled out that 13q− is related to other factors, and we found that 13q− alone does have an adverse effect on prognosis. With a higher percentage of 13q− cells, the impact on the prognosis of patients would be obvious. Finally, to further verify the accuracy of the cutoff values calculated by Kaps, we constructed prognostic models based on the results of multivariate analysis of the 2 different criteria and visualized the prognostic models by



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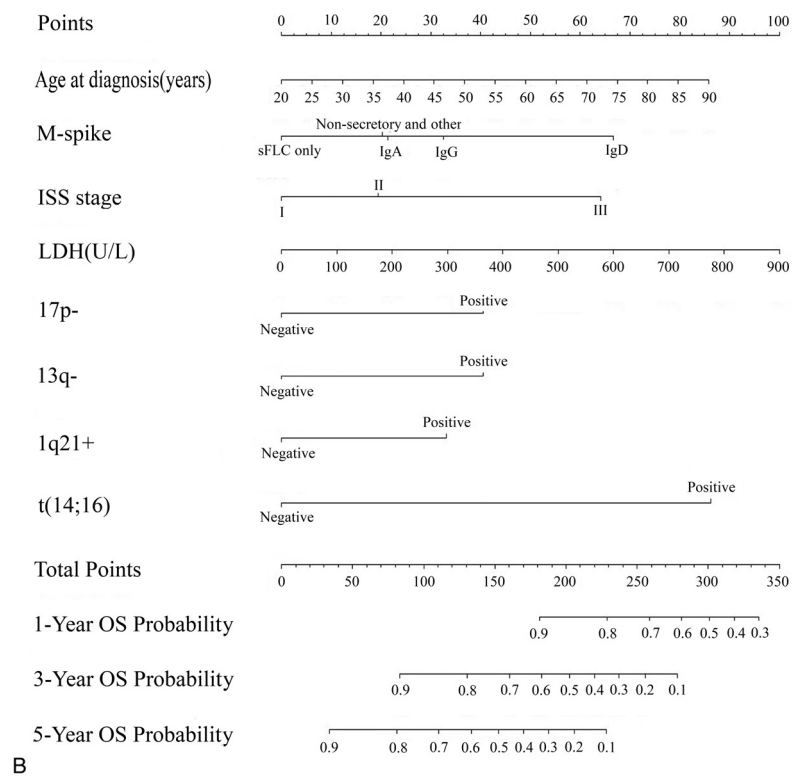

B

Figure 2. Nomograms analysis. Nomograms based on the EMN criteria predicted 1-, 3-, and 5-year overall survivals in patients with newly diagnosed MM (A), Nomograms based on the cutoff value calculated by Kaps predicted 1-, 3-, and 5-year overall survivals in patients with newly diagnosed MM (B). For each characteristic, find the position on the 0–100 scale at the top and then add these points. Find the number on the "Total Points" scale and then read the OS probabilities at the (1-, 3-, and 5-year OS probability) line of the nomogram. EMN criteria = the European Myeloma Network criteria, MM = multiple myeloma OS = overall survival.

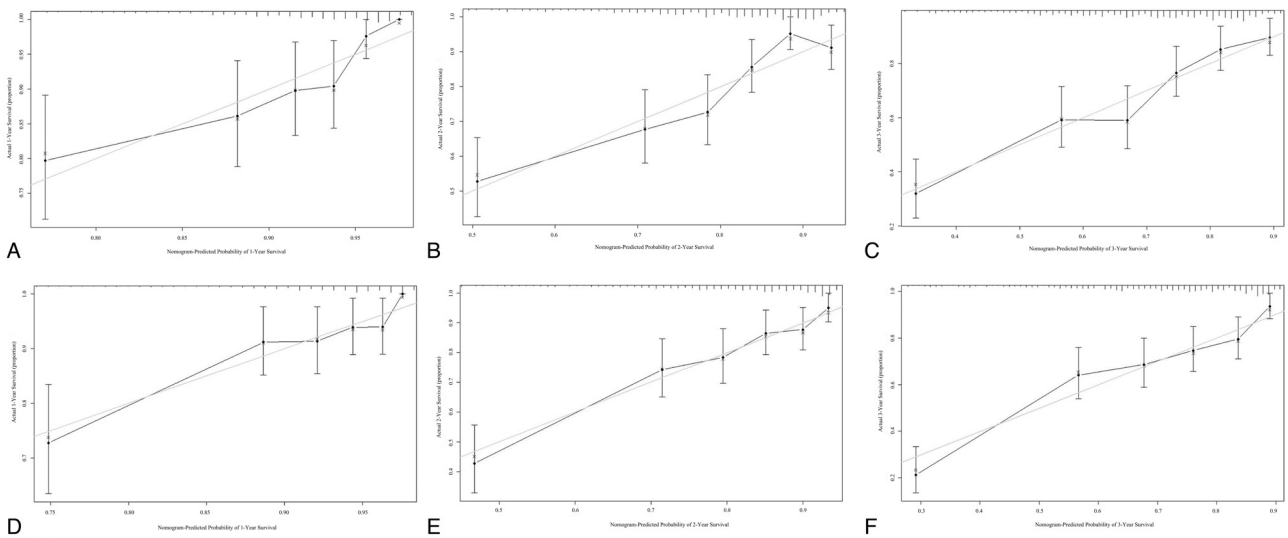

Figure 3. The calibration curves for nomograms. The calibration curves for nomograms were calculated by the EMN criteria for patients with MM predicting OS at 1, 2, and 3 years after diagnosis (A-C). The calibration curves for nomograms were established by Kaps method for patients with MM predicting OS at 1, 2, and 3 years after diagnosis (D-F). EMN criteria = the European Myeloma Network criteria, MM = multiple myeloma OS = overall survival.

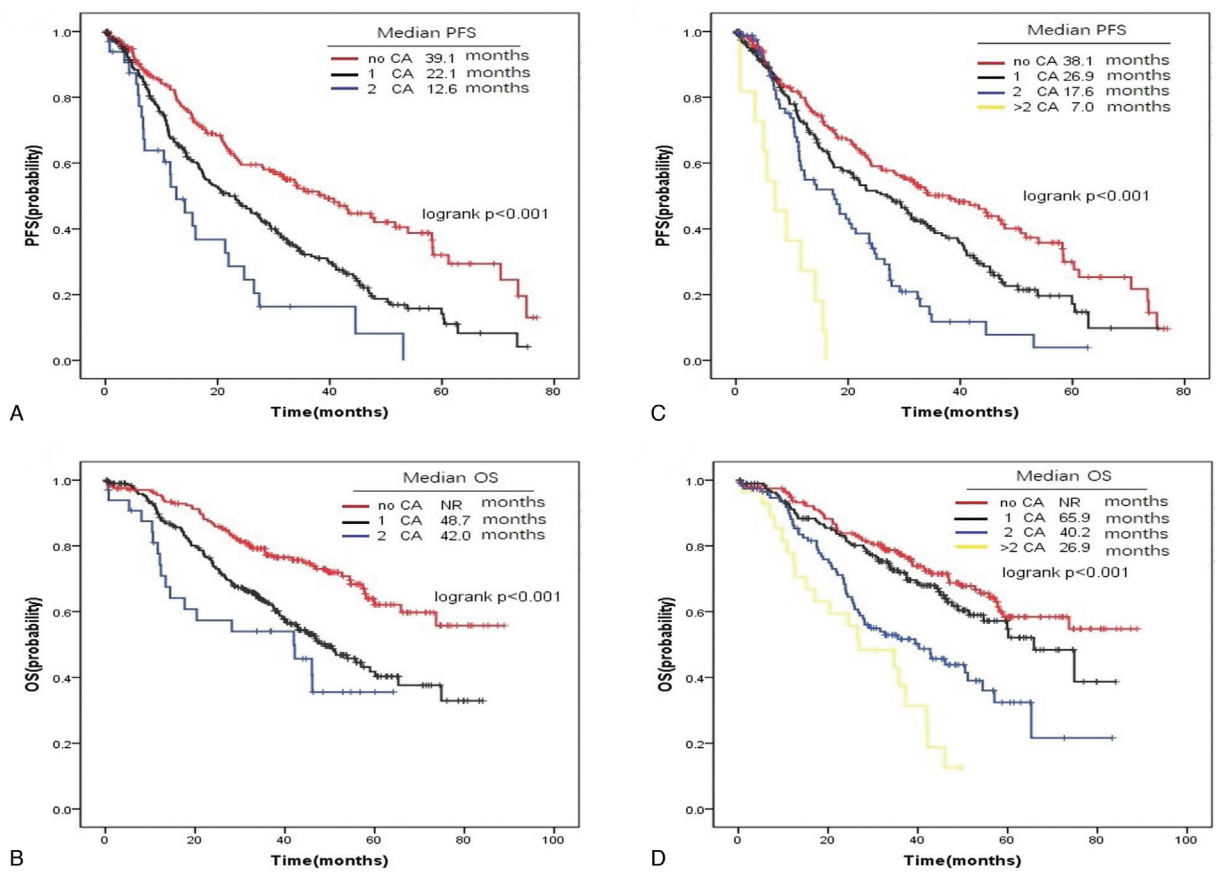

Figure 4. The impact of adverse CA. Impact of the number of adverse CA according to the EMN criteria (A-B). Impact of the number of adverse CA according to the standard calculated by Kaps (C-D). CA = cytogenetic abnormalities, EMN criteria = the European Myeloma Network criteria.

nomogram. The c-index (0.719) and internal validation corrected c-index (0.707) of the prognostic model established according to the criteria calculated by Kaps were greater than 0.7, we considered the prognostic model constructed by the Kaps method could be of medium accuracy, while the model constructed according to the EMN standard has lower accuracy. Considering MM is characterized by significant heterogeneity and it is difficult to predict the prognosis, we conclude that the prognostic model constructed by the Kaps method is more accurate in predicting the prognosis of MM patients than the EMN standard.

Finally, we analyzed the effects of adverse CA numbers on prognosis. Both univariate and multivariate analyses based on EMN criteria or criteria calculated by Kaps showed that PFS and OS in patients with greater than or equal to 1 adverse lesion had statistical differences when compared with the no abnormalities group. The HR values became larger with the greater adverse CA numbers suggested that with the increase of the number of adverse CA, the prognosis of MM patients gradually deteriorated. Therefore, lymphoma, the "double-hit theory," can be applied to MM, just like the study by Walker et al,[33] Shah et al,[31] and mSMART 3.0 updated by Mayo Clinic lately.

In summary, this study showed that 17p−, 13q−, 1q21+, and t(14;16) can lead to poor prognosis, and the proportion of adverse CA will also affect the prognosis of MM patients. In particular, the effect of 13q− on prognosis at the lower proportion is often not significant and overlooked clinically. As far as this study is concerned, the prognosis of MM patients can be more accurately predicted using the cutoff values 17p− 20.1%, 13q− 85%, 1q21 +39%, and t(14;16) 87%. Also, the more number of adverse CA indicates more deterioration of the prognosis.

There were several limitations in this study. First of all, this was only a retrospective study, not a randomized control trial. Secondly, this study was a single-center trial with a limited sample size. Thus, expanding the sample size by collaborating with other centers is preferred to study the effects of CA and other factors on the prognosis of MM patients.

4. CONCLUSION

Chromosomal abnormalities in different proportions and combinations can affect the prognosis of MM patients. Therefore, effective criteria should be formulated to evaluate the prognosis of MM patients better.

5. MATERIAL AND METHODS

5.1. Patients

From January 1, 2008 to December 31, 2015, patients with MM who were treated at Shanghai Changzheng Hospital were recruited in this study. Information about patients at the time of initial diagnosis, including age, gender, Durie–Salmon (DS) score, International Staging System (ISS) score, hemoglobin, clonal BM plasma cells, creatinine, serum calcium, β2-microglobulin, albumin, platelet, lactate dehydrogenase (LDH), bone marrow plasma cell count, FISH test results, and therapies and response status were abstracted from the patients' hospital records. All patients with MM were eligible for the 2014 International Myeloma Working Group criteria for the diagnosis of MM and were tested for CA by FISH before treatment. All participants were followed up until July 31, 2018. Patients gave written informed consents, which were performed in accordance with the Declaration of Helsinki.

5.2. FISH studies

All patients were tested for CA by FISH. The DNA probes included 1q21(CKS1B), 17p−(TP53), 13q−(D13S319), IgH probes, dual fusion probe probes t(4;14)(p16; q32)/FGFR3-IGH, t(11;14)(q13; q32)/CCND1-IGH, t(14;16)(q32; q23)/MAF-IGH, excluded t(14;20) due to the low proportion among MM patients, and other probes were used according to the product instructions. All samples were purified by anti-CD138 magnetic beads of plasma cells before FISH. OLYMPUS BX51 fluorescence microscope was used to observe the fluorescence hybridization signals of 200 interphase cells in each sample with each probe under the excitation of DAPI/FITC/RED trichrome filter. The image was analyzed by FISH analysis software (Video Test).

5.3. The main endpoints

In this study, the primary endpoint was OS which was calculated from the time of diagnosis to the time of death for any cause. The secondary endpoint was progress free survival (PFS), calculated from the time of diagnosis to the time of progression or any cause of death.

5.4. Statistical analysis

SAS Version 9.4 and R version 3.3 were used for statistical analysis. The continuous variables of normal distribution were expressed as mean ± standard deviation, the continuous variables of non-normal distribution were expressed as median (interquartile range), the categorical variables were expressed as frequency (percentage [%]). Kaps method was used to calculate the best cutoff value of OS for each CA.[6] Survival curves were compared by the Kaplan–Meier method, log-rank test. Cox proportional hazards model was used to estimate the hazard ratio (HR) along with 95% confidence intervals (95% CIs). Cox stepwise regression was used to screen variables related to OS or PFS among multiple factors. A prognostic model was constructed based on multivariate analysis results and was presented as a nomogram. The performance of the prediction model was evaluated by the calibration curve and Harrell concordance index (c-index). At the same time, cross-validation was used for internal validation and the corrected c-index was calculated. P values were 2-sided, and results were statistically significant if $P \leq .05$.

AUTHORS' CONTRIBUTIONS

T.L, W.Q, and J.L, collected and analyzed the data, and wrote the first draft, and approved the final version of the manuscript; All authors performed patient management and approved the final version of the manuscript; J.L, L.L, H.H, and H.J, performed patients' follow-up, participated in final data analysis and approval of the Title Page final version of the manuscript; W. F., and J.H. designed the study, performed patient management, and approved the final version of the manuscript, and; J.D. designed the study, performed patient management, analyzed the data, wrote the first draft, approved the final version of the manuscript.

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