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 was 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 progression 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 0.698). 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). The process of MM formation is the process of CA changing accompanying the clinical characteristics of MM patients changing. 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. The cutoff values that scholars recommended for 17p−, 1q21+, and 13q− was 60%, 30%, and 74%, respectively in the IFM 99 test, and for 1q21+, 13q− was 20% and 10%, respectively at the Mayo Center. 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...
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 (0.21–98.88) 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 impact 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) |
| Igotype                                |           |
| 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                               |           |
| I                                      | 172 (32.27%) |
| II                                     | 162 (30.39%) |
| III                                    | 199 (37.34%) |
| LDH [U/L, median [range]]              | 162 (66–819) |
| PLT×10^12/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 (45.50%) |
| 1q21+ ≥20%                             | 294 (55.16%) |
| t(11;14) ≥10%                          | 352 (66.04%) |
| t(14;16) ≥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. |

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.

### 2.3. 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 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 < 0.001] of 2 abnormalities group were shorter than that of the no abnormalities group. The survival curves were shown in Figure 2A 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 < 0.001] and 2 abnormalities group [HR, 2.811 (95% CI, 1.762–4.485), P < 0.001]. There were also 2 independent

| Table 2. | 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 < 0.001] of 1 abnormality group and the OS [HR, 2.920 (95% CI, 1.715–4.971), P < 0.001] and PFS [HR, 3.046 (95% CI, 1.948–4.762), P < 0.001] of 2 abnormalities group were shorter than that of the no abnormalities group. The survival curves were shown in Figure 2A 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 < 0.001] and 2 abnormalities group [HR, 2.811 (95% CI, 1.762–4.485), P < 0.001]. There were also 2 independent

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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/C0, 1q21+, 13q/C0, 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. 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. Therefore, it was very important to predict the prognosis of different MM patients based on their clinical characteristics. At present, the prognostic...
evaluation system for MM included the DS stage, ISS stage, R-ISS stage, mSMART stage, etc. 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. Then, when 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. The cutoff values of different chromosomes in different centers were inconsistent. For example, the cutoff value of 17p– varied from 10% to 60%. 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., 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. 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, which can be called a more accurate prediction in the real world.

### Table 2
The results of the univariate Cox regression analysis

| Characteristics | PFS | OS |
|-----------------|-----|----|
|                 | 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 |
| 17p−            | 1.606 (1.186–2.175) | .002 | 1.803 (1.258–2.582) | .001 |
| 13q−            | 1.156 (0.926–1.445) | .201 | 1.478 (1.126–1.941) | .005 |
| t(4;14)         | 1.182 (0.894–1.561) | .241 | 1.343 (0.959–1.881) | .086 |
| t(11;14)        | 1.285 (0.942–1.754) | .114 | 1.314 (0.901–1.916) | .156 |
| t(14;16)        | 1.031 (0.384–2.768) | .952 | 1.165 (0.479–2.833) | .736 |

### Table 3
Multivariate analysis results according to the EMN criteria

| Characteristics | PFS | OS |
|-----------------|-----|----|
|                 | HR (95% CI) | P value | HR (95% CI) | P value |
| 1q21+           | 1.699 (1.328–2.173) | <.001 | 1.713 (1.528–2.331) | <.001 |
| 17p−            | 1.670 (1.214–2.297) | .002 | 1.794 (1.219–2.640) | .003 |
| ISS stage       |     |    |     |    |
| I               | Reference |     |     |     |
| II              | 1.073 (0.788–1.461) | .654 | 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         |     |    |     |    |
| IgA             | Reference |     |     |     |
| 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 (0.667–1.450) | .969 | III | Reference |     |
| Non-secretory and other | 1.658 (0.748–3.678) | .213 | Isotype |     |
| Gender          |     |    |     |    |
| Male            | Reference |     |     |     |
| Female          | 0.759 (0.539–0.969) | .027 | I | 0.217 (0.030–1.561) | .129 |
| Transplantation schemes |     |    |     |    |
| Non-ASCT        | Reference |     |     |     |
| ASCT            | 0.667 (0.487–0.914) | .012 | IgG | 1.403 (0.947–2.078) | .091 |
| alloSCT         | 0.776 (0.542–1.760) | .545 | IgD | 2.197 (1.313–3.675) | .002 |

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

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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.\(^24\) In this study, among the patients with t(4;14) \( \geq 10\% \), those who underwent the chemotherapy regimens that contained bortezomib seemed to improve its adverse effects.\(^24\) In this study, the criteria according to the EMN criteria or based on 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, t(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.\(^27\) The current primary treatment regimen did not improve the prognosis of patients with t(17p).\(^35\) In this study, we calculated the t(17p) using the optimal cutoff value of 20.1% by Kaps and the results showed that t(17p) were unfavorable prognostic factors whether based on univariate analysis or multivariate analysis. Because the EMN recommended the cutoff values be relatively conservative,\(^3\) it indicated t(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 (\( \geq 4 \) 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. Therefore, 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

\[ \text{Table 4} \]

Multivariate analysis results according to the cutoff value calculated by Kaps

| Characteristics                     | PFS          | Characteristics                     | OS           |
|-------------------------------------|--------------|-------------------------------------|--------------|
|                                     | HR (95% CI)  | \( P \) value                        | 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                           |              |                                      |              |                              |
| I                                   | Reference    |                                      | I           | Reference                       |
| II                                  | 1.077 (0.792–1.456) | .638                                 | II          | 1.881 (1.411–2.508) | <.001 |
| III                                 | 1.001 (1.000–1.002) | .002                                 | III         | 1.002 (1.001–1.003) | <.001 |
| LDH                                 |              |                                      | LDH         | 1.062 (1.040–1.141) | .145 |
| Isotype                             |              |                                      | Non-secretory and other         | 1.022 (0.359–2.912) | .967 |
| IgA                                 | Reference    |                                      |              |                              |
| IgG                                 | 1.142 (0.837–1.557) | .403                                 | IgA         | 1.249 (0.847–1.840) | .262 |
| sFLC only                           | 2.044 (1.352–3.080) | <.001                                | sFLC only   | 2.245 (1.341–3.760) | .002 |
| Non-secretory and other             | 0.977 (0.668–1.429) | .903                                 | Non-secretory and other         | 0.682 (0.408–1.141) | .145 |
| Transplantation schemes             |              |                                      |              |                              |
| Non                                 | Reference    |                                      |              |                              |
| ASCT                                | 0.656 (0.479–0.900) | .009                                 |              |                              |
| alloSCT                             | 0.732 (0.323–1.656) | .454                                 |              |                              |

\( \text{alloSCT} = \text{allogeneic stem cell transplantation, ASCT} = \text{autologous hematopoietic stem cell transplantation, ISS stage} = \text{International Staging System stage, LDH} = \text{lactate dehydrogenase, OS} = \text{overall survival, PFS} = \text{prostate free survival, sFLC} = \text{serum free light chain.} \)
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.
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 (inter-quartile range), the categorical variables were expressed as frequency (percentage [%]). Kaps method was used to calculate the best cutoff value of OS for each CA. 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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