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ORIGINAL ARTICLE

Achieving minimal residual disease-negative by multiparameter flow cytometry may ameliorate a poor prognosis in MM patients with high-risk cytogenetics: a retrospective single-center analysis

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Abstract

The aim of our study was to evaluate the prognostic impact of minimal residual disease (MRD) and high-risk cytogenetics (HRCs) on outcomes in multiple myeloma (MM) patients. We applied multiparameter flow cytometry (MFC) to detect MRD in 123 consecutive patients diagnosed with MM for the first time who achieved very good partial remission (VGPR) or better after bortezomib or thalidomide-based induction therapy. Moreover, we examined the cytogenetic features of MM patients using magnetic-activated cell sorting and interphase fluorescence in situ hybridization (MACS-iFISH) at diagnosis. In all 123 MM patients, progression-free survival (PFS) and overall survival (OS) were better in the MRD− group ($n = 31$) than in the MRD+ group ($n = 92$) (median PFS: not reached (NR) vs. 26 months (m), $P = 0.0002$; 4-year OS, 91.7% vs. 66.3%, $P = 0.008$). PFS and OS were significantly shorter for each increase of one log per MRD level ($P < 0.0001$ and $P = 0.001$). The median PFS of the four groups according to the ratio of aberrant plasma cells (less than 0.01%, 0.01–0.1%, 0.1–1%, and more than 1%) were NR, 37 m, 26 m, and 15 m, respectively, and the 4-year OS rates were 91.7%, 69.3%, 76.1%, and 54.0%, respectively. In addition, our results show that PFS and OS were better for the standard-risk cytogenetic (SRC) patients than the HRC patients (median PFS: NR vs. 26 m, $P = 0.004$; 3-year OS: 95.8% vs. 76.0%, $P = 0.006$). The independent predictors of PFS were HRC and MRD+, which had hazard ratios of 1.901 (95% CI 1.094–3.303) and 3.486 (95% CI 1.449–8.386), respectively; while those for OS were an LDH level ≥ 250 U/L, HRC, and MRD+, which had hazard ratios of 2.789 (95% CI 1.080–7.199), 2.697 (95% CI 1.053–6.907), and 7.714 (95% CI 1.040–57.227), respectively. Furthermore, for SRC patients or HRC patients, PFS and OS were all longer in MRD− than in MRD+ patients. Strikingly, there was no significant difference in PFS or OS between the MRD-HRC and MRD+SRC groups (median PFS 45 vs. 34 m, $P = 0.300$; 4-year OS 100% vs. 83.6%, $P = 0.196$). PFS was superior in MRD-SRC than in MRD-HRC (NR vs. 45 m, $P = 0.035$); however, there was no significant difference in the 4-year OS between MRD-SRC and MRD-HRC (87.5% vs 100%, $P = 0.480$). MRD+ and HRCs were both independent prognostic factors in MM patients. Moreover, achieving MRD− may ameliorate a poor prognosis in MM patients with HRCs.

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Keywords Multiple myeloma · Minimal residual disease · Multiparameter flow cytometry · Cytogenetic risk stratification · Prognostic factor

Abbreviations

MFC	Multiparameter flow cytometry
BM	Bone marrow
MRD	Minimal residual disease
SRC	Standard-risk cytogenetics
HRCs	High-risk cytogenetics
HD	Hyperdiploidy
NHD	Non-hyperdiploidy
APCs	Aberrant plasma cells
NPCs	Normal plasma cells
MACS-iFISH	Magnetic-activated cell sorting and interphase fluorescence in situ hybridization
CR	Complete remission
sCR	Stringent CR
VGPR	Very good partial remission
Ig	Immunoglobulin
ISS	International Staging System
LC	Light chain
PFS	Progression-free survival
OS	Overall survival
NR	Not reached
IMWG	International Myeloma Working Group
PCR	Polymerase chain reaction
NGS	Next-generation sequencing

Background

Multiple myeloma (MM) is a clonal plasma cell malignancy that accounts for 10% of all hematological malignancies and is characterized by notable interpatient and intraclonal heterogeneity [1]. Novel agents have led to improvements in clinical outcomes for patients with MM [2–4]. However, there is currently no cure for MM, and the 5-year survival rate of MM patients is 48.5% [5]. In patients who achieve complete remission (CR), there is always minimal residual disease (MRD), and nearly all such patients eventually relapse, it is therefore necessary to search for treatments that provide deeper responses, particularly in this era of novel agents [6].

At present, highly sensitive assays, such as multiparameter flow cytometry (MFC), polymerase chain reaction (PCR), and next-generation sequencing (NGS), have been used to evaluate MRD in the treatment of MM [7–9]. Of these technologies, MFC is routinely applied in both primary diagnosis and MRD monitoring in MM and offers simultaneous identification and rapid analysis while being a cost-effective and highly sensitive approach to detect MRD [10, 11]. Recently, the International Myeloma Working Group (IMWG) has described new categories in which MRD-negative (MRD−) is considered in cases showing no evidence of myeloma in the bone marrow (BM) by MFC or NGS with a sensitivity of at least 10^{-5} in addition to negative PET/CT [12]. In addition, they emphasized the important role of MRD in the assessment of responses and recognized that using a patient's MRD status to guide treatment decisions is an approach that needs further study.

There is already evidence indicating that MRD positivity is associated with a poor prognosis [13, 14]. Patients with t(11;14), t(6;14), and a normal karyotype are standard-risk cytogenetics (SRCs) with a favorable prognosis, whereas del(17p13), t(4;14), t(14;16), t(14;20), and + 1q21 are high-risk cytogenetics (HRCs) with an adverse prognosis despite intensive treatment with induction, auto-HSCT and maintenance therapy [15–17]. However, only a few studies that have explored MRD in MM have mentioned cytogenetics [18–20]. Paiva's study showed that a patient's MRD status and HRCs were both independent factors that predicted unsustained CR, but they did not analyze the impact of the MRD status combined with HRCs on the prognosis in MM patients [20]. As far as I have known, only two studies have reported that MRD status predicts progression-free survival (PFS) and OS in patients with unfavorable cytogenetics [21, 22]; however, their results were unclear regarding subgroups analyzed by a combination of MRD status and cytogenetics, and this approach therefore needs further study.

In the present study, we evaluated MRD in 123 consecutive MM patients when they achieved very good partial remission (VGPR) or better after bortezomib or thalidomide-based induction therapy and examined their cytogenetic characteristics at diagnosis.

Subjects and methods

Patients

A total of 123 MM patients from the department of hematology, Jinling Hospital, Nanjing, China, who achieved VGPR or better after bortezomib or thalidomide-based induction therapy and had available follow-up data were enrolled in our study. The follow-up dates were from January 2011 to August 2018, and all patients provided written informed consent. Among the included patients, 65 were treated with VCD (bortezomib, cyclophosphamide, and dexamethasone; clinical trial registration no. NCT02086942), and 58 were treated with BiCTD (clarithromycin, cyclophosphamide, thalidomide, and dexamethasone; clinical trial registration no. NCT02248428) as introduction and consolidation regimens. Sixty-one patients

received maintenance therapy with cyclophosphamide (0.2 g/1–14 days) plus prednisone (60 mg/1–7 days) or no further therapy ($n = 62$) until progression at the attending physician's discretion. Treatment responses were evaluated after each cycle according to the IMWG criteria. The study protocol was approved by the ethics committees of Jinling Hospital, and the study protocol was performed in accordance with the Declaration of Helsinki.

Bone marrow samples were examined by MFC when patients achieved VGPR or better after bortezomib or thalidomide-based induction therapy. Bone marrow samples were examined by MACS-iFISH in MM patients at diagnosis. Routine clinical and laboratory parameters, including gender, age, International Staging System (ISS) stage, Durie-Salmon (DS) stage, M protein subtype in serum or urine, serum lactate dehydrogenase (LDH) level, bone marrow plasma cell (BMPC) count, immunofixation electrophoresis (IFE), and serum-free light chain (sFLC) measurement, were also performed simultaneously. For the sFLC assay, an FLC k/λ ratio (rFLC) outside the 0.26–1.65 range was considered abnormal [23].

Multiparameter flow cytometry (MFC) for MRD assessment

Bone marrow aspirates were obtained for MRD assessment when patients reached VGPR or better after induction therapy. Flow cytometry for MRD detection was performed as previously described [14, 24]. Briefly, 200 μl of an EDTA-anti-coagulated bone marrow aspirate sample was washed with PBS three times and then incubated with a 6-color antibody combination [24] that included CD38-APCS-CY7 (Biolegend, American), CD45-Pacific Blue, CD19-PE-vio770, CD56-APC, cKappa-FITC, and cLambda-PE in all cases and CD138-APC, CD38-APC-CY7, CD45-Pacific Blue, CD19-PE-vio770, cKappa-FITC, and cLambda-PE in some cases in which CD38 expression was too low to gate PC. CD38, CD45, and CD138 were purchased from Biolegend (San Diego, CA); CD19 and CD56 were obtained from Miltenyi Biotec (Germany), and cKappa-FITC and cLambda-PE were obtained from Dako (Glostrup, Denmark). The samples were gated with CD45 (− or ±)/CD38+ and CD38+/SSC for identification of PCs. To differentiate between normal and aberrant PC expression, the kappa to lambda ratio was evaluated in CD19-positive and -negative subgroups of total PCs. Patients were considered MRD-positive (MRD+) if ≥ 20 aberrant plasma cells (APCs) were detected in no less than 200,000 nucleated cells, and the sensitivity of our flow cytometry detection for MRD was 10^{-4}. Data were acquired on a MACS Quant™ (Miltenyi, Germany), and MACS Quantify™ software was used for the analysis.

Cytogenetic characterization by MACS-iFISH

Plasma cells were initially purified from bone marrow specimens obtained from all included patients using MACS with CD138 immunomagnetic microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). Subsequently, the purified and enriched plasma cells were detected by iFISH. Probes targeting RB1, D13S319, P53, 1q21, IgH, IgH/CCND1, FGFR3/IgH, and MAF/IgH (GP Medical, Beijing, China) were applied to detect del(13q14), del(17p13), +1q21, and IgH translocations, including t(14q32), t(11;14), t(4;14), and t(14;16), respectively. CEP3, CEP9, CEP11, and CEP15 (Abbott Molecular, Des Plaines, USA) were combined to detect hyperdiploidy. We defined +1q21, del(17p13), and t(14q32), including t(4;14) and t(14;16), as HRCs, and normal FISH products and other factors were defined as SRCs. Two hundred DAPI (4′,6-diamidino-2-phenylindole)-stained nuclei were counted and analyzed for each probe under a fluorescence microscope. The cutoff values were 10% for fusion and 20% for deletion or amplification according to the European Myeloma Network (EMN) recommendations [25], and trisomy of at least two of the chromosomes (3, 9, 11, and 15) ≥ 10% was defined as hyperdiploidy [26].

Statistical analysis

Statistical analyses were also performed using SPSS version 19 statistical software (Chicago, USA). Clinical and cytogenetic characteristics were compared between the two groups using Pearson's chi-square test or Fisher's test. PFS was the time between the date of diagnosis and the date of disease progression or death and was determined at the last follow-up visit. OS was calculated from the date of diagnosis until death. PFS and OS were calculated by the Kaplan-Meier method, while a log-rank test was used for comparisons. Multivariate analysis of variables associated with survival was conducted using the Cox Proportional-Hazard model for PFS and OS. A statistically significant difference was considered at $P < 0.05$.

Results

Patient and clinical characteristics

A total of 123 patients with MM were enrolled in this study. The patients' demographic and disease characteristics are summarized in Table 1. The median age was 59 years old (range, 32 to 83 years old). Of the 123 MM patients, 31 were MRD− and 92 were MRD+, as defined by MFC. Of all the patients, 72 (58.5%) were classified as having high-risk cytogenetics with del17p, 1q21, t(4;14), and/or t(14; 16) by MACS-iFISH at diagnosis. Hyperdiploidy (HD) data were

Table 1 Patient and clinical characteristics

Characteristic	Total $n = 123$	MRD positive $n = 92$	MRD negative $n = 31$	P
Gender				0.712
Male *n* (%)	67 (54.5)	51 (55.4)	16 (51.6)	
Female *n* (%)	56 (45.5)	41 (44.6)	15 (48.4)	
Median age (range)	59 (32–83)	59 (43–80)	58 (32–83)	0.338
ISS stage III *n* (%)	66 (53.7)	46 (50)	46 (50)	
Myeloma subtype				0.805
IgG *n* (%)	55 (44.7)	39 (42.4)	16 (51.6)	
IgA *n* (%)	31 (25.2)	23 (25.0)	8 (25.8)	
IgD *n* (%)	5 (4.1)	4 (4.3)	1 (3.2)	
LC *n* (%)	30 (24.4)	24 (24.4)	6 (19.4)	
Non secretory	2 (24.4)	2 (1.6)	0 (0)	
Light chain isotype				0.642
κ *n* (%)	67 (54.5)	49 (53.3)	18 (58.1)	
λ (%) *n* (%)	56 (45.5)	43 (46.7)	13 (41.9)	
LDH, median IU/L (range)	186(104–1555)	182(104–1555)	198 (107–285)	0.520
Bone lesions *n* (%)	94 (76.4)	73 (79.3)	21 (67.7)	0.188
Extramedullary mass *n* (%)	28 (22.8)	25 (27.5)	3 (9.7)	0.042
BMPC ≥ 30% *n* (%)	44 (35.8)	34 (37.0)	10 (32.2)	0.637
Bortezomib-based induction therapy *n* (%)	65 (52.8)	41 (44.6)	24 (77.4)	0.002
Maintenance therapy *n* (%)	61 (49.6)	44(47.8)	17 (54.8)	0.499
High-risk cytogenetic *n* (%)	72 (58.5)	54 (58.7)	18 (58.1)	0.557
Hyperdiploidy($n = 98$) *n* (%)	50 (51.0)	38 (51.4)	12 (50.0)	0.547

available for 98 patients, including 50 patients with HD and 48 patients with non-hyperdiploidy (NHD).

There were no significant differences between MRD+ and MRD− patients in terms of sex, age, ISS stage, myeloma subtype, light chain type, LDH, bone lesions, BMPC ≥30%, maintenance therapy, HRCs, or hyperdiploidy. However, the percentage of patients with extramedullary mass was higher in the MRD+ group than in the MRD− group (27.5% [25/92] vs. 9.7% [3/31], $P = 0.042$). A total of 52.8% (65/123) patients were treated with bortezomib as the induction therapy, and the percentage of patients treated with a bortezomib-based therapy was higher in the MRD− group than in the MRD+ group (77.4% vs. 44.6%, $P = 0.002$).

Response evaluation

After induction therapy, in 123 selected patients, we observed stringent CR (sCR) in 17.9% (22/123) of the patients, CR in 19.50% (24/123) of the patients, and VGPR in 62.60% (77/123) of the patients. These results are shown in Table 2.

In the MRD group, 51.6% (16/31) of the patients achieved sCR, 25.8% (8/31) achieved CR, and 22.6% (7/31) achieved VGPR. In the MRD+ group, 6.5% (6/92) of the patients achieved sCR, 17.4% (16/92) achieved CR, and 76.1% (70/92) achieved VGPR (Table 2). The percentages of sCR and

Table 2 The treatment responses of subgroups according to MRD status and cytogenetic risk stratification

Variables	Treatment response		
	sCR	CR	VGPR
Total *n* (%)	22 (17.9)	24 (19.5)	77 (62.6)
MRD status			
MRD− *n* (%)	16 (51.6)[a]	8 (25.8)	7 (22.6)[a]
MRD+ *n* (%)	6 (6.5)	16 (17.4)	70 (76.1)
Risk cytogenetic			
Standard risk cytogenetic *n* (%)	6 (11.8)	12 (23.5)	33 (64.7)
High-risk cytogenetic *n* (%)	16 (22.2)	12 (16.7)	44 (61.1)
MRD status and risk cytogenetic			
MRD-SRC *n* (%)	6 (46.2)[bc]	4 (30.8)	3 (23.1)[bd]
MRD-HRC *n* (%)	10 (55.6)[ef]	4 (22.2)	4 (22.2)[ef]
MRD + SRC *n* (%)	0 (0)[g]	8 (21.1)	30 (78.9)
MRD + HRC *n* (%)	6 (11.1)	8 (14.80)	40 (74.1)

MRD− compared with MRD+: [a] $P < 0.001$; MRD-SRC compared with MRD + SRC: [b] $P < 0.001$; MRD-SRC compared with MRD + HRC: [c] $P < 0.01$, [d] $P < 0.001$; MRD-HRC compared with MRD + SRC: [e] $P < 0.001$; MRD-HRC compared with MRD + HRC: [f] $P < 0.001$; MRD + SRC compared with MRD + HRC: [g] $P < 0.05$

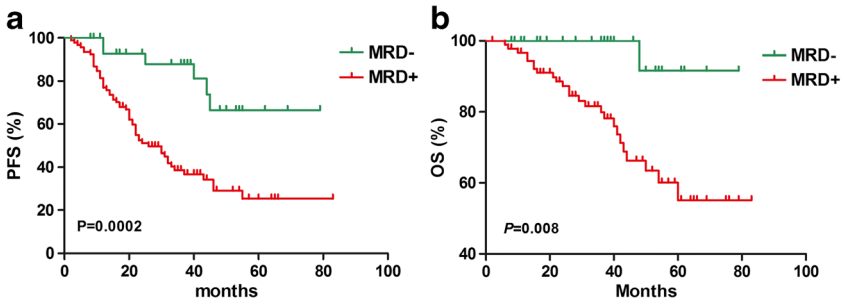

Fig. 1 PFS and OS in MM patients according to MRD status. PFS in MRD-positive and -negative groups (**a**); OS in MRD-positive and -negative groups (**b**)

CR or better was significantly higher in the MRD− group than in the MRD+ group (51.6% [16/31] vs. 6.5% [6/92], $P = 0.000$; 77.4% [24/31] vs. 23.9% [22/92], $P = 0.000$).

In the SRC group, 11.8% (6/51) of the patients achieved sCR, 23.5% (12/51) achieved CR, and 64.70% (33/51) achieved VGPR. In the HRC group, 22.2% (16/72) of the patients achieved sCR, 16.70% (12/72) achieved CR, and 61.10% (44/72) achieved VGPR (Table 2). There was no significant difference between the two groups in CR, sCR, or VGPR.

The patients were divided into four groups according to MRD status and cytogenetic risk stratification: MRD-SRC, MRD-HRC, MRD + SRC and MRD + HRC. The percentages of patient in these four groups who achieved CR plus sCR were 76.9% (10/13), 77.8% (14/18), 21.1% (8/38), and 25.9% (14/54), respectively (Table 2). There were significant differences in the sCR rate in the MRD-SRC vs. MRD + SRC comparison (46.2% vs. 0%, $P = 0.000$), the MRD-SRC vs. MRD + HRC comparison (46.2% vs. 11.1%, $P = 0.008$), the MRD-HRC vs. MRD + SRC comparison (55.6% vs. 0%, $P = 0.000$), and the MRD-HRC vs. MRD + HRC comparison (55.6% vs. 11.1%, $P = 0.000$); however, the sCR rate was lower in MRD + SRC than in MRD + HRC (0% vs. 11.1%, $P = 0.040$).

Survival analysis

We investigated the association between MRD status and both PFS and OS in MM patients. The median follow-up time was 36 months (m). Disease progression after treatment occurred in 62 patients, and 26 patients had died before the final follow-up date.

These results show that the median PFS and 4-year OS of the MRD− patients were significantly superior than those in the MRD+ patients (NR vs. 26 m, $P = 0.0002$; 91.7% vs. 66.3%, $P = 0.008$, Fig. 1). Furthermore, we investigated the impact of the MRD level on survival in all patients. Subsequently, the patients were divided into four categories according to MRD level: APC < 0.01% (MRD−, $n = 31$, group 1), 0.01% ≤ APC% < 0.1% ($n = 32$, group 2), 0.1% ≤ APC% < 1% ($n = 32$, group 3), and APC% ≥ 1% ($n = 28$, group 4). As expected, Kaplan–Meier curves revealed significant differences in PFS and OS ($P < 0.0001$ and $P = 0.001$) among the four groups. The median PFS of the four groups were NR, 37 m, 26 m, and 15 m, respectively. The 4-year OS in the four groups was 91.7%, 69.3%, 76.1%, and 54.0%, respectively. These results are shown in Fig. 2.

According to the cytogenetic risk stratification, the results showed that the median PFS and 3-year OS were better in the SRC patients than in the HRC patients (NR vs. 26 m, $P = 0.004$; 95.8% vs. 76.0%, $P = 0.006$, Fig. 3). Subsequently, we analyzed survival among the four groups according to MRD status and cytogenetic risk stratification: MRD-SRC (group1, $n = 13$), MRD-HRC (group2, $n = 18$), MRD + SRC (group3, $n = 38$), and MRD + HRC (group4, $n = 54$). Kaplan–Meier curves revealed significant differences in PFS and OS among the groups ($P = 0.0002$ and $P = 0.0006$). The best PFS and OS were observed in MRD-SRC patients, and the worst were observed in MRD + HRC patients. In SRC patients, PFS and OS were longer in MRD− than in MRD+ patients (NR vs. 34 m, $P = 0.009$; 4-year OS: 87.5% vs. 83.6%, $P = 0.508$).

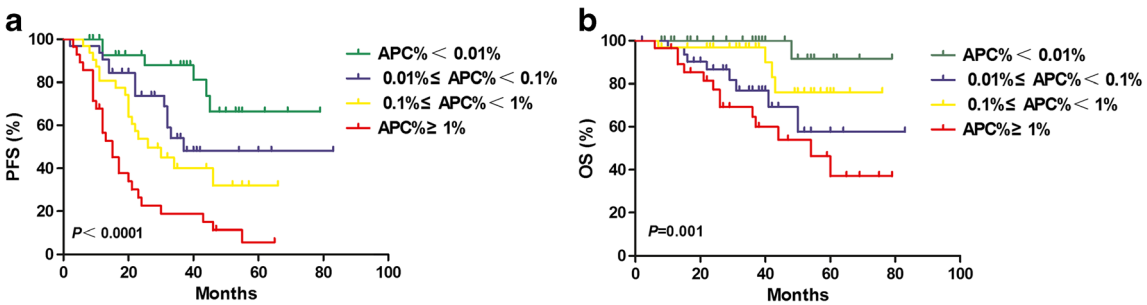

Fig. 2 PFS and OS in MM patients according to MRD level. PFS (**a**) and OS (**b**) in APC% < 0.01% (group 1, $n = 31$), 0.01% ≤ APC% < 0.1% (group 2, $n = 32$), 0.1% ≤ APC% < 1% (group 3, $n = 32$), and APC% ≥ 1% (group 4, $n = 28$)

Similar results were observed in HRC patients (45 vs. 22 m, $P = 0.016$; 4-year OS: 100% vs. 54.0%, $P = 0.012$). Strikingly, there was no significant difference in PFS or OS between the MRD-HRC and MRD+SRC groups (45 vs. 34 m, $P = 0.300$; 4-year OS: 100% vs. 83.6%, $P = 0.196$). PFS was longer in the MRD-SRC group than in the MRD-HRC group (NR vs. 45 m, $P = 0.035$), but there was no significant difference in 4-year OS between the MRD-SRC and MRD-HRC groups (87.5% vs 100%, $P = 0.480$). These results are shown in Fig. 4.

We also analyzed the prognostic impact of HD; however, there was no significant difference in median PFS or 3-year OS between the HD and NHD patients (44 vs. 25 m, $P = 0.155$; 3-year OS: 82.6% vs. 85.2%, $P = 0.464$).

Univariate and multivariate analyses of factors for PFS and OS

In the univariate analysis, BMPC ≥ 30%, HRC, and MRD+ were associated with shorter PFS, while extramedullary mass and bortezomib-based induction regimen showed trends. LDH ≥ 250 IU/L, maintenance therapy, HRC, and MRD+ were associated with shorter OS (Table 3).

In the multivariate analysis, HRC and MRD+ were independent predictors of PFS with hazard ratios of 1.901 (95% CI 1.094–3.303) and 3.486 (95% CI 1.449–8.386), respectively. An LDH level ≥ 250 U/L, HRC, and MRD+ were independent predictors of OS with hazard ratios of 2.789 (95% CI 1.080–7.199), 2.697 (95% CI 1.053–6.907), and 7.714 (95% CI 1.040–57.227), respectively. However, while maintenance therapy did not predict PFS, it showed a trend for OS, with hazard ratios of 0.478 (95% CI 0.212–1.075; $P = 0.074$) (Table 3).

Discussion

The detection of MRD has emerged as a significant tool in the management of myeloma since it has become viewed as highly important for evaluating the response in MM and is strongly associated with PFS and OS [12, 27]. MRD assessment techniques, such as MFC, PCR, and NGS, although not all are yet routinely available, have the potential to achieve a high level of sensitivity. Compared to MFC, a disadvantage of molecular-based MRD methodologies is the necessity for initial clone identification when the disease burden is high, which limits its application [7–9]. NGS is highly sensitive, but it is relatively time-consuming and requires a sample to be obtained at the initial diagnosis [9]. MFC does not require a diagnostic sample and is less time-consuming. Thus, MFC is a rational, cost-effective, and highly sensitive approach to detect MRD in routine clinical practice. At present, no standardized MRD panel is used for MM in clinical practice. Some scholars recommend that MRD detection of MM should use more than eight color-MFC panels [28, 29]. However, different medical institutions do not currently use the same the MRD panels.

Although overall outcomes for myeloma patients are improving in the novel agent time, that those with HRD still had worse outcomes compared to patients with SRD [15–17]. Previous studies confirm that MRD positive is a poor prognostic factor for the PFS or/and OS [14, 21, 30]. However, only a few studies that have explored MRD in MM have mentioned cytogenetics, so we analyzed the prognosis of MM patients according to the MRD and cytogenetic risk stratification [18–20]. Thus, we analyzed the outcome of MM patients by a combination of MRD status and cytogenetics.

Our study showed that of all 123 MM patients who achieved VGPR or better after induction therapy, 31 were MRD− and 92 patients were MRD+ by MFC. There were no significant differences between MRD+ and MRD− patients in clinical characteristic parameters, such as ISS stage or the cytogenetic risk profile. However, extramedullary mass was more frequently observed in the MRD+ group than in the MRD− group. The percentage of patients who received bortezomib-based treatment was higher in the MRD− patients than in MRD+ patients. However, MRD status was verified as an independent prognostic marker in our study. Consistent with our result, de Tute RM's results [31] showed that the prognostic impact of MRD was independent of the induction

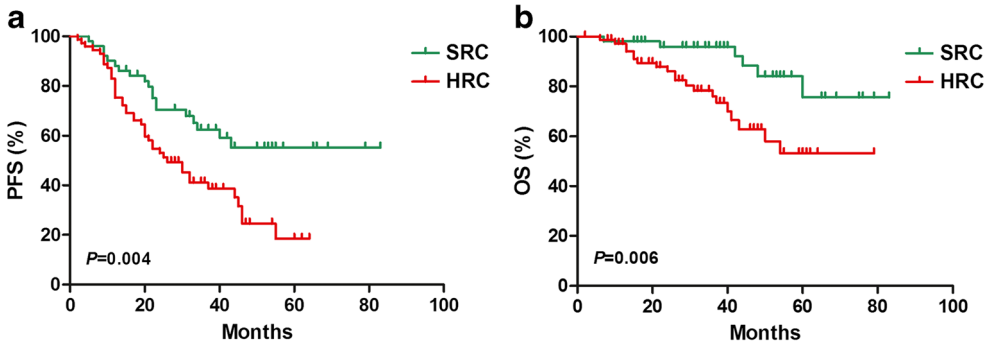

Fig. 3 PFS (**a**) and OS (**b**) in MM patients according to cytogenetic risk status

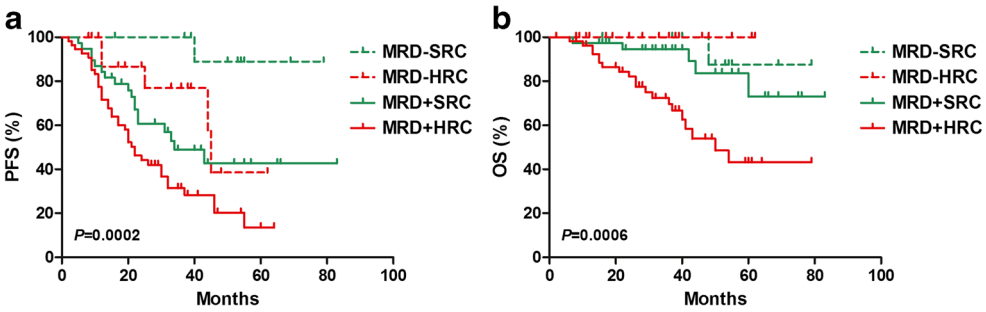

Fig. 4 PFS (**a**) and OS (**b**) in MM patients according to combined cytogenetic risk and MRD status: MRD-SRC (group 1, $n = 13$), MRD-HRC (group 2, $n = 18$), MRD + SRC (group 3, $n = 38$), and MRD + HRC (group 4, $n = 54$)

therapy received; however, the patients in their study had not received bortezomib-based treatment and instead received autologous stem cell transplant (ASCT).

The sCR plus CR rates observed in our study after induction therapy were similar to those reported in previous studies [32, 33]. The percentage of CR or better was significantly higher in the MRD− group than in the MRD+ group. However, 22.6% of the MRD− patients achieved only VGPR. This contradictory phenomenon was also reported in previous studies [13, 34]. Rawstron et al. [13] showed that 26% (34/246) of MRD− patients did not achieve conventional CR, including 12% (29/246) who had less than VGPR. This result may correspond to the late serologic responders or false-negatives due to immunophenotyping because of persistent clonal PCs outside the BM or in a BM area for which the sample obtained was not representative. There was no significant difference between the SRC and HRC groups in the rate of CR, sCR, or VGPR.

PFS and OS were worse in the MRD+ group than in the MRD− group. While some previous studies have shown that MRD is a predictor of PFS but not OS [18, 35, 36], others have found that MRD is a prognostic factor for both PFS and OS, consistent with our findings [14, 21, 30]. Munshi et al. showed in their meta-analysis that achieving an MRD− status was associated with a significant improvement in PFS and OS [27]. We therefore further divided all patients into four categories according to MRD level. As expected, the survival analysis showed that PFS and OS significantly shorten for each increase of one log by MRD level (NR, 37 m, 26 m, and 15 m for PFS, $P < 0.0001$; 91.7%, 69.3%, 76.1%, and 54.0% for 4 year-OS, $P = 0.001$). Although there was no difference in OS between groups 2 and 3 according to MRD

Table 3 Univariate and multivariate analyses of factors and their relationship with PFS and OS

Variables	PFS				OS			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR 95% CI	*P*	HR 95% CI	*P*	HR 95% CI	*P*	HR 96% CI	*P*
Gender (male vs. female)	1.059 0.641–1.747	0.824			1.465 0.665–3.229	0.344		
Age (≥ 65 vs. < 65)	0.864 0.489–1.527	0.615			0.914 0.383–2.179	0.839		
ISS (III vs. I or II)	1.040 0.632–1.712	0.877			1238 0.571–2.682	0.588		
BMPC (≥ 30 vs. < 30)	1.684 1.014–2.795	0.044	1.614 0.937–2.779	0.084	1.033 0.460–2.322	0.937		
LDH (≥ 250 vs. < 250)	1.724 0.818–3.635	0.152			3.533 1.406–8.874	0.007	2.789 1.080–7.199	0.034
Bone lesions (yes vs. no)	1.726 0.876–3.398	0.114			0.840 0.336–2.098	0.709		
Extramedullary mass (yes vs. no)	1.649 0.949–2.865	0.076	1.388 0.789–2.440	0.255	1.780 0.763–4.125	0.182		
Bortezomib-based induction regimen (yes vs. no)	0.645 0.390–1.067	0.088	0.801 0.467–1.374	0.420	0.943 0.436–2.038	0.881		
Maintenance therapy (yes vs. no)	0.716 0.461–1.255	0.285			0.444 0.199–0.990	0.047	0.478 0.212–1.075	0.074
High Cytogenetic risk (yes vs. no)	2.166 1.285–3.728	0.005	1.901 1.094–3.303	0.023	3.365 1.343–8.431	0.010	2.697 1.053–6.907	0.039
Hyperdiploidy (yes vs. no)	0.672 0.385–1.174	0.162			1.403 0.564–3.492	0.466		
MRD status (positive vs. negative)	4.235 1.818–9.862	0.001	3.486 1.449–8.386	0.005	9.117 1.234–67.388	0.030	7.714 1.040–57.227	0.046

level (4-year OS: 69.3% vs. 76.1%, $P = 0.116$), there was a trend toward a shorter OS as the MRD level increased. Our results indicate that in MM patients, PFS and OS are associated with the MRD level, consistent with previous studies that have used either MFC or allele-specific oligonucleotide (ASO)-PCR [14, 18, 35, 36]. Rawstron AC's study demonstrated that the MRD level, when viewed as a continuous variable determined by FCM, independently predicted both PFS and OS with an approximate 1-year median OS benefit per log depletion [14]. Martinez-Lopez et al. also noted that MRD levels assessed by NGS can be quantitative as a better median PFS was predicted by deeper levels of MRD (MRD ≥ 10^{-3}, PFS of 27 m, MRD 10^{-3} to 10^{-5}: PFS of 48 m and MRD < 10^{-5}: PFS of 80 m) [37]. Thus, the MRD level should be considered when evaluating a prognosis in MM.

Our results indicate that MRD+ and HRCs are independent prognostic factors of PFS and OS, consistent with previous studies [14, 18, 21, 38]. Our study further illustrates that PFS and OS are longer in both HRC and SRC patients who are MRD− than in those who are MRD+. Thus, achieving MRD− may be associated with superior outcomes even in patients with HRCs. This result was consistent with those presented in previous studies in which MRD was detected by MFC or NGS [21, 38]. Strikingly, there was no significant difference in PFS between the MRD-HRC and MRD+SRC groups in our study, and there were no significant differences in OS between the MRD+SRC and MRD-HRC, MRD-SRC and MRD-HRC groups, these results indicating that achieving MRD− may overcome the poor prognosis associated with certain adverse cytogenetic risk factors identified at diagnosis. Chakraborty R's study analyzed 185 myeloma patients with high-risk disease who underwent upfront ASCT and found that those who achieved an MRD− status at day 100 after transplantation had a median PFS that was superior to that obtained in those who remained MRD+ (26 vs. 17 m, $P < 0.001$) [22]. However, that study did not analyze an MRD combined with SRC group. A recent study showed that HRD patients who achieved MRD+ at 3 m post-auto-HSCT had a median PFS of 19.0 m, while this median PFS was not reached in HRD patients who were MRD−, and the best OS was seen in SRD patients who were MRD− at the last follow-up [21]. However, in their study, there was only a trend toward a longer OS in MRD-negative patients ($P = 0.14$) [21], and they did not explore the differences in outcomes between the MRD + SRC and MRD-HRC groups. Therefore, our study may provide a basis for a more precise prognostic stratification of MM patients in which MRD status is recognized as playing an important role in prognostic evaluation.

Maintenance therapy improves PFS among patients with MM [39, 40]. However, in our study, maintenance therapy did not predict PFS but did show a trend for OS. Fukumoto's study showed that maintenance therapy after CR was an independent predictor of PFS and OS in MM patients who achieved immunophenotypic CR [41]. Hence, MRD may aid in prognostication and guide the type and duration of maintenance therapy in MM patients in the near future.

In our study group, recently proven HD was a good prognostic factor, the coexistence of which may ameliorate an adverse prognosis in MM patients with HRCs [42]. However, there was no significant difference in PFS or OS between the HD and NHD groups in this study. This was probably because the patients enrolled in our study included only those who achieved VGPR or better after induction therapy and not newly diagnosed MM patients, who were included in previous reports [43, 44]. Thus, additional studies are required to confirm our results.

Our study has several limitations. First, MRD detected by FCM was only sensitive to the degree of 10^{-4} as this was the accepted standard when we began this study, and the whole study lasted for a long period of time. If we had changed the MRD panel midway, there would have been differences in detection sensitivity. In addition, the follow-up time was short, and the number of subjects was small in our study.

Conclusion

This retrospective study reveals the MRD status and cytogenetics of MM patients and their outcomes in a single center in China. We found that PFS and OS were significantly longer in MM patients with MRD− than in those with MRD+. Our results demonstrate that MRD+ and HRCs are independent prognostic factors in MM patients. Achieving MRD− may ameliorate a poor prognosis in MM patients with HRCs. Furthermore, MRD and MRD levels may be used as risk factors for the prognosis of MM patients in the future and to direct accurate personal therapy. Considering the limitations of our study, these findings should be confirmed by trials that contain large samples and more sensitive MFC.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This study was approved by the Clinical Research Ethics Committee of Jinling Hospital, Nanjing, China. Written consent was waived by the Ethics Committee.

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