Prognostic value of circulating plasma cells in newly diagnosed multiple myeloma

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Abstract

Purpose: Multiple myeloma (MM) is a clinically and biologically heterogeneous malignancy of plasma cell. The overall survival of MM patients varies from people to people ranged from several months to decades. It is always knotty how to predict MM prognosis. The presence of circulating plasma cells (CPCs) has been associated with a worse prognosis in patients with MM.

Materials and Methods: This study retrospectively analyzed CPCs in 108 cases of newly diagnosed MM patients with 8-color flow cytometry to investigate its value for outcome prediction, and combined CPCs with R-ISS to stratify the risk of MM.

Results: CPCs were detected in 58/108 patients (53.7%). The optimum cutoff predicting for overall survival was determined as 0.105% by using a ROC analysis. Compared with patients with CPCs < 0.105% (n = 66, 61.1%), those with CPCs ≥ 0.105% (n = 42, 38.9%) showed lower blood platelet count (BPC) ($P=0.038$), but higher β 2-microglobulin (β 2-MG), lactate dehydrogenase (LDH), ferritin (FER), and harboring P53 deletion, high-risk cytogenetic abnormality, ($P = 0.011, 0.001, 0.002, <0.001$, and 0.020 , respectively). The higher R-ISS stage seems to harbor higher CPCs. CPCs ≥ 0.105% are independently factor for adverse outcome ($P < 0.001$). The combination of R-ISS staging system and CPCs level was used to stratify the risk of multiple myeloma and R-ISS III stage with CPCs ≥ 0.105% was ranked as a real ultra-high-risk group.

Conclusion: This study suggests that high CPCs is associated with an aggressive disease and that the current R-ISS system in conjunction with CPCs may facilitate the differentiation of NDMM patients. There may also be the potential significance in modifying the definitions of high-risk disease and the practice of adopting a risk-adapted initial treatment.

Introduction

Multiple myeloma (MM) is the second common hematologic cancer, characterized by malignant proliferation of abnormally cloned plasma cells in the bone marrow (BM) and eventually other tissues. The introduction of proteasome inhibitors (PIs), immunomodulators (IMiDs), histone deacetylase inhibitors and monoclonal antibodies has improved the clinical outcome of multiple myeloma in the last decade^[1], however, there is still a group of patients who possess poor survival and resistance to novel agents. Therefore, it is necessary to screen out these high-risk patients and provide them with more intensive regimen. The definition of high-risk group has been evolving consistently, however it is still controversial. The most widely accepted definition, which included the revised International Staging System (R-ISS)^[2] and the recently updated mSTART (Mayo Stratification for Myeloma And Risk-adapted therapy), based on cytogenetic and clinical biomarkers. In the latest study, Walker BA et al described a high-risk type of myeloma through genome analysis as the double-hit myeloma which involved patients with either TP53 double allele mutation or ISS stage III with CKS1B (1q21+), accounting for 6.1% of the total number of patients.^[3] Unfortunately, Next-generation sequencing is expensive and time and labor consuming. It is essential for prognostic factor assays to be applied in a feasible and economic way.

Higher circulating clonal plasma cells (CPCs), identified by cytology^[4], multiparameter flow cytometry^[5], or slide-based immunofluorescence^[6] is associated with a poor prognosis in patients with MM^[5, 7] and is also a risk factor of progression in patients with monoclonal gammopathy of undetermined significance^[8] and smoldering MM^[9]. Two studies have proposed that the cutoff value for plasma cell leukemia (PCL) should be revised to 5% CPCs on

peripheral blood smear as its similar outcomes to originally defined PCL (i.e., $\geq 20\%$ CPCs)^[10–12]. Although the biological significance of CPCs in MM patients still remains unknown, CPCs represent a unique subset of patient-paired potential clonogenic BM PCs, which have a quiescent phenotype^[13]. Therefore, we hypothesize that the quantification of CPCs could have implications for redefining high-risk disease. Moreover, if CPCs is incorporated into the current powerful prognostic system R-ISS stage, a much higher-risk group which is more aggressive will be distinguished. In the current study, we retrospectively analyzed circulating clonal plasma cells in 108 newly diagnosed multiple myeloma (NDMM) patients and investigated its correlation with other clinical parameters and survival. We also combined CPCs with R-ISS staging system to better stratify MM patients into subgroups of homogeneous survival.

Patients And Method

Patients

108 newly diagnosed MM patients between October 2015 and June 2018 were included. Patients were required signing informed consent, which were performed in accordance with the Declaration of Helsinki and approved by the hospital ethics committees. Clinical and laboratory data were collected as followings: age, sex, immunoglobulin classification, ISS stage and other clinical data such as $\beta 2$ -MG, albumin, C-reactive protein (CRP), lactate dehydrogenase (LDH) levels, percentage of bone marrow PC.

The diagnostic criteria and response evaluation were based on IMWG criteria^[12]. Patients were followed up for their treatment response, progression-free survival (PFS) and overall survival (OS).

CPC tests by Multi-parameter Flow Cytometry

CPCs was assessed by 8-color flow cytometry. Specimens were prepared with red blood cell lysis within 24 hours after collection, and then stained with specific antibodies combination (CD38 PBE/CD56 A750/CD19 PerCP-Cy5.5/CD138 APC/CD45 KO/CD27 Pe-Cy7/ κ FITC/c λ PE), and evaluated by flow cytometry. All samples were analyzed using FACSCantoII flow cytometry instrument with FACSDiva software (BD Biosciences). The clonal CPCs were defined as cytoplasmic light chain ratio (Kappa: lambda > 3.0 or < 0.3). The target for collection was > 500000 cellular events. The clonal CPCs rate was calculated as the number of clonal PCs/ 500000 total mononuclear cells. Patients were defined to be negative for clonal CPCs at a sensitivity of ~ 1 in $10,000$ clonal plasma cells in all tested events.

Fluorescence *in situ* hybridization analyses

The Fluorescence in situ hybridization study was performed in 98/108 patients at initial diagnosis, using bone marrow samples, ten of which were not assessed because of inadequate samples. Five chromosomal abnormalities including t (4; 14), t (14; 16), 1q21 amplification, IgH rearrangement and P53 deletion were detected by interphase fluorescence in situ hybridization (iFISH) on CD138 sorted plasma cell. Totally 100 to 300 interphase nuclei in each sample were scored, as appropriate. The positive cutoff threshold was set as 20% for P53 deletion and 1q21 amplification, 10% for immunoglobulin H (IgH) translocations. High risk cytogenetic abnormalities include t (4; 14), t (14;16) and P53 deletion.

Statistical analysis

Mann–Whitney U test was used to compare differences between continuous variables, and Chi-square tests and Fisher exact tests for nominal variables. The correlations between CPCs and patients' clinical stages were

calculated by Spearman correlational analysis. Distribution of OS and PFS were plotted by Kaplan-Meier (K-M) curves and log-rank test was applied to estimate differences between groups. Univariable and multivariable analysis of survival were carried out by Cox regression model. GraphPad Prism 8.0 (GraphPad Software, San Diego, CA) and SPSS (version 25.0) software (IBM Corporation, Armonk, NY, USA) were used for statistical analysis. All reported P-values were two-sided, with confidence intervals of 95%, and P-value ≤ 0.05 was considered statistically significance. Before the log-rank test, CPCs optimal cutoff values was determined by the receiver operating characteristic (ROC) curves.

Results

Clinical characteristics

The median age of patients (n = 108) was 63 (range, 36–84) years, of which sixty-seven (62%) were males. All patients recruited from Oct. 2015 to Jun. 2018 were followed up until Sep. 2019 and their median follow-up time was 22 (range, 2.0–43.0) months. CPCs were detected in 58/108 patients (53.7%). No circulating PCs were found in the remaining 50 patients (46.3%). The eligible cutoff value for predicting overall survival was 0.105% with a sensitivity of 69% and a specificity of 75% by using a ROC analysis. Based on this, the patients were divided into two groups, one of which involved in 42 (38.9%) patients with CPCs $\geq 0.105\%$, another involved 66 (61.1%) patients. The clinical parameters of the two groups were compared. Patients with CPCs $\geq 0.105\%$ had higher levels of $\beta 2$ -microglobulin ($P = 0.011$), lactate dehydrogenase (LDH) ($P = 0.001$), ferritin (FER) ($P = 0.002$), and more likely to harbor P53 deletion ($P < 0.001$), in addition to lower Blood platelet count (BPC) level ($P = 0.038$), compared with those with CPCs $< 0.105\%$. The characteristics of these two groups are shown in Table 1.

Table 1
Clinical characteristics of the 108 newly diagnosed MM patients according to CPCs expressions

Clinical characteristics	All patients (n = 108)	Patients with CPCs < 0.105% (n = 66)	Patients with CPCs ≥ 0.105% (n = 42)	P-value
Age (yrs)	62(36–84)	63 (36–84)	63 (43–79)	0.808
Male (No,0%)	67(62%)	39(59.1%)	28(66.7%)	0.429
β2-MG (mg/L)	5.89(0.22–59.8)	4.73 (0.22–35.90)	7.07 (1.69–59.8)	0.011
ALB (g/L)	33.45(13.5–50.2)	32.75(13.5–50.2)	33.95 (16.3–49.6)	0.373
LDH (U/L)	181(67–825)	159 (67–356)	216.5 (84.0-825)	0.001
HB (g/L)	85(40–146)	86.5 (40–146)	83.5 (50–125)	0.494
BPC (× 10 ⁹ /L)	157(30–349)	166.5 (30–507)	144.5 (41–349)	0.038
sCr (μmol/L)	82(35.4–1194)	84.7(38.4–1194)	79.1 (35.4–932)	0.922
ESR (mm/H)	105(5-145)	117.5 (5-145)	80 (5-140)	0.288
CRP (mg/L)	3.27(2.0-79.8)	3.16 (3.02-56)	3.27 (2.0-79.8)	0.336
FER (μg/L)	305.2(5-1500)	232.3 (5-1500)	431.2 (76.7–1500)	0.002
Bone marrow PC (%)	18.9(1.2–92.4)	17.6 (1.2–88.8)	23.2 (5.2–92.4)	0.052
EMD (n,%)	19(17.6)	11 (16.7)	8(18.2)	0.751
FISH (available on 98pts)				
Deletion P53(%)	14(14.3)	2(3.4)	12(30)	< 0.001
1q21amplification (%)	51(52.0)	30(51.7)	21(52.5)	0.940
t(4,14)(%)	14(14.3)	9(15.5)	5(12.5)	0.675
t(14,16)(%)	2(2.0)	1(1.7)	1(2.5)	0.789
IgH translocations (%)	24(24.5)	13(22.4)	11(27.5)	0.565
High-risk cytogenetic abnormality (%)	29(29.6)	12(20.7)	17(42.5)	0.020
Initial therapy				
Novel agents	102(94.4%)	64(97%)	38(90.5%)	0.383

Abbreviations: β2-MG, β2-microglobulin; ALB, Serum albumin; LDH, Lactate dehydrogenase; HB, Hemoglobin; BPC, Blood platelet count; sCr, Serum creatinine; ESR, Erythrocyte sedimentation rate; CRP, C-reactive protein; FER, Ferritin; FISH, fluorescent in-situ hybridization; High-risk cytogenetics was defined by del(17p), t(4;14), t(14;16).

Clinical characteristics	All patients (n = 108)	Patients with CPCs < 0.105% (n = 66)	Patients with CPCs ≥ 0.105% (n = 42)	P-value
Bortezomib-based	61(56.5%)	40(60.6%)	21(50%)	0.278
IMiD-based	37(34.3%)	21(31.8%)	16(38.1%)	0.539
Thalidomide	27(25%)	15(22.7%)	12(28.6%)	0.494
Lenalidomide	10(14.8%)	6(9.1%)	4(9.5%)	0.940
Bortezomib + IMiD	4(3.7%)	3(4.6%)	1(2.4%)	0.561
Conventional chemotherapy	6(5.5%)	2(3.0%)	4(9.5%)	0.151
Abbreviations: β2-MG, β2-microglobulin; ALB, Serum albumin; LDH, Lactate dehydrogenase; HB, Hemoglobin; BPC, Blood platelet count; sCr, Serum creatinine; ESR, Erythrocyte sedimentation rate; CRP, C-reactive protein; FER, Ferritin; FISH, fluorescent in-situ hybridization; High-risk cytogenetics was defined by del(17p), t(4;14), t(14;16).				

The correlations between clinical stages and CPCs levels

16.7%(1/6) of patients with CPCs ≥ 0.105% were in DS stage I, 31.6% (6/19) in DS stage II and 42.2% (35/83) in DS stage III ($P = 0.232$). In terms of ISS stage, the proportions of each stage were 23.1% (3/13) 34.2% (13/38) and 45.6% (26/57) ($P = 0.092$). In 98 patients available for R-ISS stratification, the proportions of each stage were 20% (2/10), 35.2% (19/54), and 55.9% (19/34) ($P = 0.017$). Although all three staging systems showed a rising trend with CPCs value, only the R-ISS staging system showed statistical significance, as shown in Table 2.

Table 2
Correlations between levels of CPCs and patients' clinical stages

Correlations between levels of CPCs and patients' clinical stages									
	DS stages			ISS stages			R-ISS stages		
	I	II	III	I	II	III	I	II	III
All patients	6	19	83	13	38	57	10	54	34
Patients with CPCs < 0.105%	5(83.3%)	13(68.4%)	48 (57.8%)	10 (76.9%)	25 (65.8%)	31 (54.4%)	8 (80%)	35 (64.8%)	15 (44.1%)
Patients with CPCs ≥ 0.105%	1(16.7%)	6(31.6%)	35 (42.2%)	3 (23.1%)	13 (34.2%)	26 (45.6%)	2 (20%)	19 (35.2%)	19 (55.9%)
P-value	0.232			0.092			0.017		
Abbreviations: DS, Durie-Salmon staging system; ISS, International Staging system; R-ISS, Revised International Staging System									

Survival analysis according to CPCs levels

At the end of follow-up time, 76 of 108 patients still survived, which made this study not reaching its' median OS. The median PFS of the whole cohort was 23.0 (95% CI: 17.9–28.2) months. The median OS of patients with CPCs $\geq 0.105\%$ was 24.0 (95%CI:15.0–33.0) months while OS was not reached for patients with CPCs $< 0.105\%$. According to K-M curves, patients with CPCs $\geq 0.105\%$ showed a significant inferior trend for survival (HR 4.73, 95%CI: 2.29–9.79; $P < 0.001$). On the other hand, the median PFS of patients in 2 groups were 16 (95% CI: 13.8–18.2) months and 35.5 (95% CI: 19.6–51.4) months, respectively (HR 2.84, 95%CI: 1.58–5.11; $P < 0.001$). All the differences had statistically significance, as shown in Fig. 1.

Initial induction treatment of MM was based on patients' characteristics, the risk of toxicity, patients' tolerance to treatment, and also the patients' willingness. Of all the patients, 61 (56.5%) patients received bortezomib-based regimens as the first-line treatment, 37 (34.3%) patients received IMiDs (thalidomide or lenalidomide) based therapy, 4(3.7%) patients received bortezomib and IMiD based therapy, and rest of 6 (5.56%) patients received conventional chemotherapy.

The Median OS of patients in both CPCs $\geq 0.105\%$ and CPCs $< 0.105\%$ groups received IMiDs-based therapy was not reached, while the median OS of patients received bortezomib-based therapy were 17.0 (95% CI: 5.6–28.4) months and not reached respectively. However, according to K-M curves, patients with CPCs $\geq 0.105\%$ showed inferior trend for survival compared to patients with CPC $< 0.105\%$ after bortezomib-based therapy (HR 8.054, 95%CI: 2.68–24.24; $P < 0.001$ Figure 2A), Interestingly, this trend was not showed significant difference in patients accepting IMiDs-based therapy (HR 2.21, 95% CI : 0.67–7.25, $P = 0.190$ Figure 2C).

On the other hand, median PFS of CPCs $\geq 0.105\%$ and CPCs $< 0.105\%$ patients received bortezomib therapy were 15.5 (95% CI:6.19–24.8) months and not reached, which had statistically significance (HR 3.39, 95%CI: 1.44–7.94; $P < 0.001$; Figure 2B) whereas in 2 groups of patients received IMiDs regimens was 19 (95% CI: 11.37–26.62) months and 35.5 (95% CI: 15.9–55) months, respectively (HR 1.38, 95%CI: 0.57–3.36; $P = 0.455$, Fig. 2D).

Survival analysis of CPCs level response after treatment

A total of 45 patients had complete follow-up data of CPCs level. Among them, 36 patients turned negative when the best response was achieved after treatment. The rest of 9 patients remained positive. The median OS of CPCs-positive patients was only 10 (95%CI 9.03–10.97) months, while that of CPCs-negative patients after treatment was not reached. It suggested inferior outcome in CPCs-positive patients (HR 10.53, 95%CI:1.85–60.1; $P < 0.001$). Median PFS of patients in 2 groups was respectively 8.0 (95% CI 5.49–12.74) and 24 (95%CI 19.37–28.63) months, which also had a significant difference (HR 4.03, 95%CI:1.12–14.58; $P < 0.001$), as shown in Fig. 3.

We further compared the minimal residual disease (MRD) status of bone marrow by flow cytometry and peripheral blood CPCs in 45 patients at remission point. MRD were still positive in bone marrow in 16 out of 36 CPCs-negative patients, while CPC can be detected in all of the patients with positive MRD in bone marrow. The negative predictive value of CPCs for bone marrow MRD was 56%, and the positive predictive value was 100% (Table 3).

Table 3
Comparison of CPCs expression and paired MRD of bone marrow

	CPCs positive	CPCs negative	Total
Bone marrow MRD positive	9	16	25
Bone marrow MRD negative	0	20	20
Total	9($PPV_{CPCs}=100\%$)	36($NPV_{CPCs}=56\%$)	45
Abbreviations: PPV_{CPCs} : positive predictive value MRD assessed from CPCs in peripheral blood; NPV_{CPCs} : negative predictive value MRD assessed from CPCs in peripheral blood			

Univariate And Multivariate Cox Analysis For CPCs

In order to evaluate whether other factors could affect prognosis other than the CPCs expression, we used a Cox proportional hazard model including age, $\beta 2$ -MG, LDH, sCr, CPCs, R-ISS stages III and High-risk cytogenetics to explore their effects on OS and PFS. As a result, we found that the following factors negatively affected OS in the univariate Cox model: $\beta 2$ -MG ≥ 3.5 mg/L, LDH ≥ 271 U/L, sCr ≥ 177.8 μ mol/L, CPCs $\geq 0.105\%$, R-ISS stages III and High-risk cytogenetics. $\beta 2$ -MG ≥ 3.5 mg/L, sCr ≥ 177.8 μ mol/L and CPCs $\geq 0.105\%$ retained their significant impact on PFS. In a multivariate analysis, only CPCs $\geq 0.105\%$ and R-ISS stages III had statistical significance for inferior OS, whereas only CPCs $\geq 0.105\%$ predicted for worse PFS (Table 4)

Table 4
Univariate and multivariate COX analysis of factors predicting worse OS and PFS

Variable	OS		PFS					
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Age ≥ 65 years	1.67(0.84–3.31)	0.141	NA	NA	1.07(0.63–1.82)	0.791	NA	NA
β2-MG ≥ 3.5 mg/L	5.51(1.32–23.05)	0.019	2.40(0.54–11.72)	0.251	2.17(1.06–4.43)	0.034	1.56(0.74–3.31)	0.242
LDH ≥ 271U/L	3.30(1.48–7.34)	0.003	1.22(0.38–3.92)	0.744	2.17(1.06–4.43)	0.092	NA	NA
ALB < 35 g/L	3.30(1.48–7.34)	0.680	NA	NA	4.43	0.374	NA	NA
sCr ≥ 177.8μmol/L	0.87(0.44–1.72)	0.005	NA	0.126	1.85(0.90–3.78)	0.027	NA	0.064
Bone marrow PCs ≥ 30%	0.87(0.44–1.72)	0.064	1.92(0.83–4.40)	NA	0.79(0.47–1.33)	0.293	1.75(0.97–3.16)	NA
CPCs ≥ 0.015%	2.79(1.37–5.67)	<0.001	NA	<0.001	1.91(1.08–3.39)	0.001	NA	0.003
Extramedullary lesions	1.91(0.96–3.80)	<0.001	NA	NA	3.39	0.833	2.30(1.32–4.01)	NA
		<0.001	4.52(2.13–9.59)	<0.001	1.91(1.08–3.39)	0.076	NA	NA
		<0.001	1.497(0.45–4.96)	0.516	1.34(0.78–2.32)	0.080	NA	NA
		<0.001			2.47(1.44–4.24)		NA	
		<0.001			0.93(0.47–1.84)			
		<0.001			1.66(0.95–2.90)			
R-ISS stages III	4.81(2.28–10.14)	<0.001			1.68(0.94–2.98)			
High-risk cytogenetics	1.22(0.53–2.81)							

Abbreviations: β2-MG, β2-microglobulin; ALB, Serum albumin; LDH, Lactate dehydrogenase; sCr, Serum creatinine; FISH, fluorescent in-situ hybridization; R-ISS, revised International Staging System; Bold entries signify statistically significant variables; NA, not applicable

Variable	OS				PFS			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
	4.97(2.35–10.49)							
	4.25(2.05–8.82)							
Abbreviations: β 2-MG, β 2-microglobulin; ALB, Serum albumin; LDH, Lactate dehydrogenase; sCr, Serum creatinine; FISH, fluorescent in-situ hybridization; R-ISS, revised International Staging System; Bold entries signify statistically significant variables; NA, not applicable								

Combined CPCs level and R-ISS stages to stratify MM patients

R-ISS staging system in conjunction with CPCs expression was applied to stratify the risk of multiple myeloma. On the background of R-ISS stage I (n = 10), there was no difference in OS between two groups with CPCs $\geq$ 0.105% and CPCs < 0.105% (NR vs. NR, $P = 0.593$). Among patients with R-ISS stage II (n=54), the median OS was 27 months for those with CPCs $\geq$ 0.105% but was not reached for patients with CPCs < 0.105% (HR 3.46, 95%CI: 0.90-13.32; $P = 0.0383$). A statistically significant difference was found for OS among R-ISS III (n = 34) between patients with CPCs $\geq$ 0.105% and CPCs < 0.105% (11.5months vs. 33 months, HR 6.09,95%CI:2.47–15.01, $P < 0.001$). There seem to be a trend for worse survival in the CPCs $\geq$ 0.105% within R-ISS stage II than that in the CPCs < 0.105% within R-ISS stage II, but showed no significant difference, as shown in Fig. 4A (27 months vs. 33 months, HR 1.99, 95%CI: 0.54–7.35; $P = 0.313$).

On the other hand, there was no difference in PFS between patients with CPCs $\geq$ 0.105% and CPCs < 0.105% which were marked as R-ISS stage I (21 months vs. NR; HR 1.041, 95%CI: 0.46–2.37; $P = 0.921$) and R-ISS stage II (13.75 months vs. NR; HR 1.97, 95%CI:0.122–31.92; $P = 0.547$). The median PFS for those with CPCs $\geq$ 0.105% was significantly worse than those patients with CPCs < 0.105% (10 months vs not reached, HR 7.32, 95%CI: 3.09–17.30; $P < 0.001$) within R-ISS stage II. but There was no significant difference in survival between patients with CPCs < 0.105% and CPCs $\geq$ 0.105%, as shown in Fig. 4B, although a trend for better survival appeared in R-ISS III stage patients with CPCs < 0.105% by comparing to all of R-ISS stage II patients or those R-ISS stage II with CPCs $\geq$ 0.105% (NR vs.21 months, HR 0.35, 95%CI: 0.12–1.03, $P = 0.052$; NR vs. 13.75 months, HR 0.26, 95%CI: 0.01–10.03, $P = 0.188$, respectively).

Discussion

Several studies have demonstrated the higher CPCs is a negative prognostic factor in multiple myeloma at several clinical investigations (diagnosis^[5], pre-stem cell transplantation^[14], and in the relapsed or refractory setting^[7]). This study has focus on 108 Chinese patients with symptomatic MM. We considered 0.105% was eligible cut-off value for CPCs in predicting poor prognosis. Newly diagnosed multiple myeloma (NDMM) cases with high clonal CPCs and its long term persistence is associated with inferior survival. Its prognostic value was independent of other well-established prognostic markers,such as high-risk FISH cytogenetics, LDH at diagnosis, β 2-MG. Interestingly, when we combined R-ISS staging system and CPCs levels to restratify the risk of multiple myeloma, patients with low

CPCs in stage I had similar outcome to that in stage II. However, high-risk R-ISS stage with CPCs $\geq 0.105\%$ was accounted as a much higher-risk factor.

The main cause leading to the existence of high number of CPCs is cytogenetic abnormalities. Recent study showed that 13q deletion and t(4;14) were more common in patients with CPCs $\geq 2\%$ compared with intramedullary MM^[4]. Another study from Mayo Clinic had also shown the incidence of t(4;14) was significantly higher in those with CPCs prior to autologous stem cell transplantation^[14]. In the present study, It was observed that patients with CPCs $\geq 0.105\%$ had a twofold risk of harboring high-risk cytogenetics compared to those with CPCs $< 0.105\%$. When assessing the frequency of individual cytogenetic abnormalities, the frequency of P53 deletion, was accounted for 14.3% of the whole cohort in the higher CPCs group, which was over eightfold (30% vs 3.4%) by comparing with the CPCs lower. Inactivation of TP53 by coding mutation or 17p13 deletion are strikingly common in both primary plasma cell leukemia(pPCL) and secondary leukemic transformation (sPCL) (pPCL 56%; sPCL 83%) [15], suggesting that the impairment of the p53 tumor suppressor pathway might have a role in extramedullary tumor expansion. As CPCs have been shown to be unique cytogenetic subclones of BMPCs, paired cytogenetic analysis of CPCs and BMPCs should be performed in large cohorts of patients to identify the driver cytogenetic abnormalities. This may shed light on their pathogenesis and role in disease progression.

With the advancement in technology utilized for diagnosis and the emergence of new agents for MM, these previously defined prognostic markers have evolved with time^[2, 16]. In this study, patients in high CPCs had a worse median OS than those stratified by R-ISS I (24 months versus 32 months) but an identical median PFS of 16 months. Of note, the CPCs $\geq 0.105\%$ was correlated with higher R-ISS stage; moreover, in R-ISS stage I patients, about half of them with CPCs $< 0.105\%$ had not inferior survival as we had believed and only those with CPCs $\geq 0.105\%$ fell into a much higher-risk group with a dire prognosis (median OS = 11.5 months; PFS = 10 months). In consideration of the strong impact resulting from multivariate COX analysis on OS, the presence of CPCs $\geq 0.105\%$ may be considered as an additional factor for R-ISS staging system. The combination of R-ISS and CPCs could facilitate the clinical diagnosis and treatment in screening out higher-risk MM patients and determining therapy regimen.

The impact of CPCs on prognosis was also evaluated by comparing OS between variant therapy regimens. We found that the prognosis of patients with high CPCs in bortezomib group was significantly inferior to that with low CPCs. However, this evidence was not found in the IMiD group. Besides, a similar proportion of CPCs positive patients turned CPCs negative after these two regimens. The above suggested IMiD was insufficient to improve the prognosis of patients with higher CPCs. Due to the relatively small cohort, it is need a large scale study and long-term follow-up to judge whether CPCs could be taken as a response factor for treatment regimen.

Interestingly, circulating plasma cells were not detected in 64% patients with MRD positive bone marrow, however there was a concomitance between CPCs positive MRD. It also indicated the positive value of CPCs as predictive factor.

This study further explains the value of CPCs as a prognostic marker in myeloma. Furthermore, the presence of CPCs appeared to enhance the current R-ISS staging prognostic system in distinguishing a subset of patients with a particularly poor prognosis. Thus, these findings may have significance in modifying the definitions of high-risk disease and clinical practice in adopting a risk-adapted initial treatment.

Conclusion

This study further explains the value of CPCs as a prognostic marker in myeloma. Furthermore, the presence of CPCs appeared to enhance the current R-ISS staging prognostic system in distinguishing a subset of patients with a particularly poor prognosis. Thus, these findings may have significance in modifying the definitions of high-risk disease and clinical practice in adopting a risk-adapted initial treatment.

Abbreviations

MM: multiple myeloma; CPCs: circulating plasma cells; ROC: receiver operating characteristic; BPC: blood platelet count; β 2-MG: β 2-microglobulin; ALB: albumin; HB: Hemoglobin; sCr: Serum creatinine; ESR: Erythrocyte sedimentation rate; LDH: lactate dehydrogenase; FER: ferritin; NDMM: newly diagnosed multiple myeloma; BM: bone marrow; PIs: proteasome inhibitors; IMiDs: immunomodulators; R-ISS: Revised International Staging System; mSTART: Mayo Stratification for Myeloma And Risk-adapted therapy; PCL: plasma cell leukemia; CRP: C-reactive protein; iFISH: interphase fluorescence in situ hybridization; IgH: immunoglobulin H; OS: overall survival; PFS: progression free survival; K-M: Kaplan-Meier; BPC: Blood platelet count; DS: Durie-Salmon staging system; ISS: International Staging System; CI: Confidence interval; HR: Hazard ratio; MRD: minimal residual disease; NR: not reached; pPCL: primary plasma cell leukemia; sPCL: secondary leukemic transformation; BMPCs: bone marrow plasma cells; PPV_{CPCs}: positive predictive value MRD assessed from CPCs in peripheral blood; NPV_{CPCs}: negative predictive value MRD assessed from CPCs in peripheral blood

Declarations

Ethical Approval and Consent to participate

The study was conducted in accordance with the principles of the Declaration of Helsinki principles and was approved by the Ethics Committee at Jiangsu Province Hospital.

Consent for publication

Not applicable.

Availability of supporting data

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

WMH, YYJ performed the study, analyzed the data and wrote the manuscript; SSZ helped with performing the 8-color flow cytometry. QLS and MX helped with collecting clinical data. XYQ and RZ helped with analyzed the data.

YJW and LJC performed the study and revised the manuscript. JYL supervised study. All authors read and approved the final manuscript.

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References

- [1] Rajkumar SV. Multiple myeloma: 2016 update on diagnosis, risk-stratification, and management. *Am J Hematol*. 2016. 91(7): 719-34.
- [2] Palumbo A, Avet-Loiseau H, Oliva S, Lokhorst HM, Goldschmidt H, Rosinol L, et al. Revised International Staging System for Multiple Myeloma: A Report From International Myeloma Working Group. *J Clin Oncol*. 2015. 33(26): 2863-9.
- [3] Walker BA, Mavrommatis K, Wardell CP, Ashby TC, Bauer M, Davies F, et al. A high-risk, Double-Hit, group of newly diagnosed myeloma identified by genomic analysis. *Leukemia*. 2019. 33(1): 159-170.
- [4] An G, Qin X, Acharya C, Xu Y, Deng S, Shi L, et al. Multiple myeloma patients with low proportion of circulating plasma cells had similar survival with primary plasma cell leukemia patients. *Ann Hematol*. 2015. 94(2): 257-64.
- [5] Nowakowski GS, Witzig TE, Dingli D, Tracz MJ, Gertz MA, Lacy MQ, et al. Circulating plasma cells detected by flow cytometry as a predictor of survival in 302 patients with newly diagnosed multiple myeloma. *Blood*. 2005. 106(7): 2276-9.
- [6] Witzig TE, Gertz MA, Lust JA, Kyle RA, O'Fallon WM, Greipp PR. Peripheral blood monoclonal plasma cells as a predictor of survival in patients with multiple myeloma. *Blood*. 1996. 88(5): 1780-7.
- [7] Gonsalves WI, Morice WG, Rajkumar V, Gupta V, Timm MM, Dispenzieri A, et al. Quantification of clonal circulating plasma cells in relapsed multiple myeloma. *Br J Haematol*. 2014. 167(4): 500-5.
- [8] Kumar S, Rajkumar SV, Kyle RA, Lacy MQ, Dispenzieri A, Fonseca R, et al. Prognostic value of circulating plasma cells in monoclonal gammopathy of undetermined significance. *J Clin Oncol*. 2005. 23(24): 5668-74.
- [9] Gonsalves WI, Rajkumar SV, Dispenzieri A, Dingli D, Timm MM, Morice WG, et al. Quantification of circulating clonal plasma cells via multiparametric flow cytometry identifies patients with smoldering multiple myeloma at high risk of progression. *Leukemia*. 2017. 31(1): 130-135.
- [10] Ravi P, Kumar SK, Roeker L, Gonsalves W, Buadi F, Lacy MQ, et al. Revised diagnostic criteria for plasma cell leukemia: results of a Mayo Clinic study with comparison of outcomes to multiple myeloma. *Blood Cancer J*. 2018. 8(12): 116.
- [11] Kumar S, Paiva B, Anderson KC, Durie B, Landgren O, Moreau P, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. *Lancet Oncol*. 2016. 17(8): e328-e346.

- [12] Granell M, Calvo X, Garcia-Guiñón A, Escoda L, Abella E, Martínez CM, et al. Prognostic impact of circulating plasma cells in patients with multiple myeloma: implications for plasma cell leukemia definition. *Haematologica*. 2017. 102(6): 1099-1104.
- [13] Paiva B, Paino T, Sayagues JM, Garayoa M, San-Segundo L, Martín M, et al. Detailed characterization of multiple myeloma circulating tumor cells shows unique phenotypic, cytogenetic, functional, and circadian distribution profile. *Blood*. 2013. 122(22): 3591-8.
- [14] Chakraborty R, Muchtar E, Kumar SK, Jevremovic D, Buadi FK, Dingli D, et al. Risk stratification in myeloma by detection of circulating plasma cells prior to autologous stem cell transplantation in the novel agent era. *Blood Cancer J*. 2016. 6(12): e512.
- [15] Tiedemann RE, Gonzalez-Paz N, Kyle RA, Santana-Davila R, Price-Troska T, Van Wier SA, et al. Genetic aberrations and survival in plasma cell leukemia. *Leukemia*. 2008. 22(5): 1044-52.
- [16] Greipp PR, San MJ, Durie BG, Crowley JJ, Barlogie B, Bladé J, et al. International staging system for multiple myeloma. *J Clin Oncol*. 2005. 23(15): 3412-20.

Figures

Figure 1

Kaplan-Meier plots according to CPCs group for overall survival (A) and progression-free survival (B)

Figure 2

Kaplan-Meier plots according to CPCs group with different treatment regimens (A: overall survival with Bortezomib-based treatment; B: progression-free survival with Bortezomib-based treatment; C: overall survival with IMiD-based treatment; D: progression-free survival with IMiD-based treatment)

Figure 3

Kaplan-Meier plots according to CPCs group after treatment for overall survival(A) and progression-free survival(B)

Figure 4

Kaplan-Meier plots according to R-ISS stages based on CPCs expression for overall survival(A) and progression-free survival(B)