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ORIGINAL ARTICLE

Prognostic impact of circulating plasma cells in patients with multiple myeloma: implications for plasma cell leukaemia definition

Running title: Circulating plasma cells in myeloma

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ABSTRACT

The presence of circulating plasma cells in patients with multiple myeloma is considered a marker for highly proliferative disease. In the present study, the impact of circulating plasma cells assessed by cytology on survival of patients with multiple myeloma was analysed. Wright-Giemsa stained peripheral blood smears of 482 patients with newly diagnosed myeloma or plasma cell leukaemia were reviewed and patients were classified in four categories according to the percentage of circulating plasma cells: 0%, 1-4%, 5-20% and plasma cell leukemia with the following frequencies: 382 (79.2%), 83 (17.2%), 12 (2.5%) and 5 (1.0%) respectively. Median overall survival according to the circulating plasma cells group was 47, 50, 6 and 14 months, respectively. At multivariate analysis, presence of 5 to 20% circulating plasma cells was associated with a worse overall survival (relative risk 4.9, 95%CI 2.6-9.3) independently of age, creatinine, Durie-Salmon and international stage. Patients with ≥5% circulating plasma cells had lower platelet counts (median $86x10^9$/L vs. $214x10^9$/L, $p<0.0001$) and higher bone marrow plasma cells (median 53% vs. 36%, $p=0.004$). The presence of ≥5% circulating plasma cells in patients with multiple myeloma has similar adverse prognostic impact as plasma cell leukemia.

Word count: 191

Key words: Multiple myeloma, cytology, prognostic factors, plasma cell leukaemia, survival.

INTRODUCTION

Plasma cell leukaemia (PCL) was originally defined by the presence of both >20% circulating plasma cells (PC) and an absolute count >2 × 10^9/L PC[1,2] although in many studies the presence of only one of the above criteria was required[3-5]. PCL may be classified as primary when it presents *de novo* in patients without previous evidence of multiple myeloma (MM) or secondary when is presented as a leukemic transformation of a previously recognized MM. Primary PCL is a rare entity with an incidence of 2% to 4% of MM[6-8] and it is associated with a worse prognosis than MM. Its median survival, in a large epidemiological study, was only four months[7]. However, with the use of novel drugs upfront, median survival ranging from 18 to 36 months has been reported[9-15].

The presence of circulating PC, identified by cytology[16], multiparameter flow cytometry[17,18] or slide-based immunofluorescence[19] is associated with a worse prognosis also in myeloma patients not fulfilling the criteria of PCL. Presence of circulating PC is also a risk factor of progression to active disease in patients with monoclonal gammopathy of undetermined significance[20] and smouldering MM[21]. It has been suggested that MM patients with circulating PC, even below 20%, could have the same bad prognosis as patients with PCL. Indeed, a lower cut-off of ≥5% or ≥0.5 x10^9/L of nucleated peripheral blood cells to redefine PCL have been proposed[3]. In the present study, the impact of the presence of circulating PC assessed by cytology on survival of patients with MM was analysed.

METHODS

Requirements to enter the study were a diagnosis of symptomatic MM or primary PCL following the IMWG criteria[22] between January 2008 and December 2013 in five University Hospitals from Catalonia, and to have peripheral blood smears at diagnosis available for review. The study was approved by the Ethic Committee of the Hospital de la Santa Creu i Sant Pau and was conducted according to the declaration of Helsinki. Clinical data including age, sex, myeloma isotype, percentage of bone marrow plasma cells, LDH, Durie-Salmon and ISS stages, as well as initial treatment and follow up, were collected from medical records. Cytogenetic analysis was performed according to local policies and patients were treated according to local protocols. Wright-Giemsa stained peripheral blood smears were reviewed by five experienced haematologists on peripheral blood cytology. A minimum of 100 nucleated cells per smear were systematically counted. Each sample was analysed by a single morphologist following the same criteria.

The primary endpoint was overall survival (OS) measured from the date of diagnosis to the date of death or last follow up. Differences in demographics and baseline characteristics were compared using two sided Fisher exact test for categorical variables and Mann-Withney U test for continuous variables. Survival analysis was performed using the Kaplan and Meier method and differences were tested for statistical significance using the log-rank test. Multivariate analysis was conducted using Cox proportional hazards model. All calculations were performed using the software SPSS® statistics version 22.

RESULTS

Clinical characteristics

The study cohort includes four hundred eighty-two patients diagnosed with MM between January 2008 and December 2013. The median age at diagnosis was 69 years (range 28 to 92 years). Two hundred sixty (53.9%) of the patients were males. The median follow up was 28 months for the whole cohort and 38 months for the patients alive. Two hundred thirty-one (47.9%) patients died during follow up. First line therapy was based on bortezomib combinations in 230 (47.7%) patients, alkylating agents with glucocorticoids in 114 (23.7%) patients, VAD or VAD-like chemotherapy in 60 (12.4%), immunomodulatory-based combinations in 26 (5.4%), high-dose dexamethasone in 4 (0.8%), and only palliative care in 48 (9.9%) patients. One hundred fifty-six (32.4%) patients received autologous stem cell transplantation as part of their first line treatment. Twelve (2.5%) patients received allogeneic stem cell transplantation during the course of the disease.

Clinical characteristics at diagnosis according to the circulating PC group are summarized in Table 1. Differences in age, gender, myeloma isotope, LDH, Durie-Salmon and ISS stages between the four groups were not statistically significant. However, patients within the 5 to 20% circulating plasma cell group had lower platelet counts (median $86x10^9$/L vs. $214x10^9$/L, $p<0.0001$) and higher proportion of bone marrow PC (median 53% vs. 36%, $p=0.004$).

The 5 patients with >20% circulating PC were initially treated with bortezomib and dexamethasone (2 patients); bortezomib, cyclophosphamide and dexamethasone (1 patient); melphalan and prednisone (1 patient); and bortezomib, melphalan and prednisone (1 patient). Of the 12 patients with 5-20% circulating PC, one died the day after the diagnosis and only received supportive care; the remaining 11 patients were initially treated as follows: bortezomib and dexamethasone (6 patients); bortezomib, thalidomide and dexamethasone (2 patients), VAD (1 patient), bortezomib, melphalan and prednisone (1 patient) and cyclophosphamide and steroids (1). One patient with >20% circulating PC and three patients with 5-20% circulating PC received an autologous stem cell transplantation as consolidation.

Risk factors for overall survival in the overall cohort

According to the percentage of circulating PC, four groups were considered for the analysis of survival: No circulating plasma cells, 382 (79.2%) patients; 1 to 4% circulating plasma cells, 83 (17.2%) patients; 5 to 20% circulating plasma cells, 12 (2.5%) patients; and classical PCL group (>20% or $>2x10^9$/L plasma cells), 5 (1%) patients. A patient with 15% circulating plasma cells but an absolute circulating plasma cell count of $2.7x10^9$/L was included in the classical PCL group.

Median OS of patients with no circulating PC, 1 to 4%, 5 to 20% and >20% were 47 (95%CI 38.6-55.4) months, 50 (95%CI 31.0-68.9) months, 6 (95%CI 0.9-11.1) months and 14 (95%CI 9.7-18.3) months, respectively (Figure 1) ($p<0.001$).

In the univariate analysis, the other factors associated with a worse survival together with circulating PC were: age older than 65 years at diagnosis, creatinine >2 mg/dL, treatment with new drugs upfront (proteasome inhibitors or immunodulatory drugs) Durie-Salmon and ISS advanced stages. Cytogenetic was not included in the survival analysis due to the lack of such data in most patients. The five patients with classical PCL were excluded of the multivariate analysis. The finding of 5 to 20% circulating plasma cells, age older than 65 years, Durie-Salmon III, creatinine >2 mg/dL, and ISS 3 retained their significance in the multivariate analysis (Table 2).

Risk factors for overall survival in patients treated with novel agents upfront

Two hundred sixty five of the 482 (54.9%) patients were treated upfront with proteasome inhibitors or inmunomodulatory drugs. Of them, 192 (72.5%) patients had 0% circulating PC, 61 (23.0%) patients had 1 to 4% circulating PC, 9 (3.4%) patients had 5 to 20% circulating PC and 3 (1.1%) patients had a diagnosis of classical PCL. One hundred ten (41.5%) patients treated with novel agents upfront died during follow up and their median OS was 50 (95% CI 38 – 61).

In patients treated with novel agents upfront, median OS in cases with 0%, 1 to 4%, 5 to 20% and PCL were 58 (95% CI NR) months, 60 (95% CI 33 – 86) months, 22 (95% CI 0 – 65) months and 14 (95% CI 1 – 26) months respectively. When only two groups were considered, <5% and ≥5% circulating PC, median OS were 58 (95% CI 46 – 69) months and 14 (95% CI 0.4 – 27) months (Figure 2).

Together with the percentage of circulating PC, the other factors associated with a

worse OS in univariate analysis were creatinin >2mg/dL and ISS stage II or III. A trend was observed in patients >65 years old and Durie Salmon stage III. Of them, only to have ≥5% circulating PC maintained statistical significance in the multivariate analysis (Table 3). LDH was not included in the multivariate analysis due to the lack of statiscal significance or even a trend in univariate analysis (RR 1.1, 95% CI 0.7 – 1.9, P= 0.467).

DISCUSSION

This study aimed to address the impact of circulating PC on survival of patients with MM. Seventeen per cent of patients had between 1% and 4% circulating plasma cells. That finding was not associated with other clinical characteristics and had no impact on survival. Completely different picture was for the 2.5% of patients with 5% to 20% circulating PC. Such patients had lower platelet counts, higher bone marrow infiltration and, importantly, a shorter survival independent of other clinical known prognostic factors. In fact, the median OS of 6 months observed in these patients is closer to that of patients with the "classical" definition of PCL. When the analysis was restricted to patients treated with novel agents upfront, the impact of circulating PC was consistent with the whole cohort. The differences in OS observed between patients with 5-20% circulating PC and >20% circulating PC (6 vs. 14 months) may be explained by the low number of cases in both groups.

Although the presence of circulating PC has been previously associated with survival, conventional cytology has been used for their assessment only in one study[16]. In that case, patients with circulating plasma cells constituted 14.1% of the overall series and

had a median survival of 25 months. The results of the present study are consistent with the ominous prognosis impact of peripheral blood plasmocytosis; however, the definition of high-risk group found was different; ≥2% circulating PC in An et al. study and ≥5% circulating PC in the present.

Using multiparameter flow cytometry, the presence of circulating PC has also been associated with survival[17,18,23]. In the study from Mayo Clinic[17], 24% of patients had more than 400 circulating PC. Such patients had a median OS of 32 months versus not reached in patients with 400 or less circulating PC. In a study from the same institution, but in this case using slide-based immunofluorescence microscopy for plasma cell quantification[19], it could be identified a 54% of patients with >4% PC; who had a median survival of 2.4 years compared to 4.4 years in patients with fewer circulating PC. The aforementioned studies used techniques much more sensitive than the present; this may explain the different percentages of patients with circulating PC identified in these studies in comparison with the present one.

Despite these findings, the definition of PCL has been based on standard morphological exam of peripheral blood[3]. In comparison with flow cytometry and immunofluorescence, conventional cytology identifies a smaller number of patients with an extremely poor prognosis. Additionally, conventional cytology has the advantage of being a simple and inexpensive technique that can be applied in any clinical laboratory worldwide. As limitation, conventional cytology is not able to identify the clonality of PC, instead of flow cytometry and immunofluorescence. This may hampered the specificity of conventional cytology since polyclonal reactive PC may be rarely detected in some patients with MM[24,25].

The presence of t(4;14), del(17p), amp(1q21) and del(1p21) in malignant PC are adverse prognostic factors in MM[26,27]. Indeed, adverse cytogenetics together with ISS 3 and/or high LDH identifies a group of patients with ultra-high risk MM[28]. Several of these genetic abnormalities, particularly del(17p)[9] and chromosome 1 alterations[29] are more frequent in PCL than in MM. In the series herein presented, del(17p) by FISH was observed in 1 of 7 patients with 5% to 20% circulating PC and 1 of 2 patients with >20% circulating PC. A limitation of the present study is that, due to the lack of cytogenetic data in most patients, the prognostic impact of unfavourable cytogenetic abnormalities and the revised-ISS[28] could not be analysed.

As highlighted in the last consensus by IMWG[3], the diagnosis of PCL has been classically done on the basis of the presence of >20% circulating PC and/or an absolute count >2 × 10^9/L PC. However, lower peripheral blood PC counts, as showed in our study (that is, ≥5% peripheral blood plasma cells) should be considered as diagnostic criteria of PCL ("PCL-like" myeloma or early PCL), due to the independent and strong prognostic impact. Prospective multicenter analysis with translational correlative studies into the biology of these patients is encouraged, as well as risk-oriented therapeutic strategies[30,31]. For the same reason, careful examination of peripheral blood by conventional microscopy should be done in all patients with MM in the daily clinical practice.

In conclusion, the presence ≥5% circulating PC by conventional cytology easily identifies a group of myeloma patients with a prognosis as poor as PCL, suggesting that the diagnosis of PCL should be revisited. If confirmed in other series, especially in

uniformly treated prospective studies, such patients may benefit from a distinct and more intensified therapeutic approach.

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AUTORSHIP

MG, JS, JB and CF designed the study, analysed the data and wrote the paper. XC, CM, MT, TG and LA reviewed peripheral blood smears. AG, LE, EA, AS, PS, DC, AV and LR collected clinical data. All the authors reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors have no competing interests.

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Table 1. Clinical characteristics according to the number of circulating plasma cell group

Circulating plasma cells	0%	1-4%	5-20%	>20%
N (% overall)	382 (79.2)	83 (17.2)	12 (2.5)	5 (1.0)
Male, n (%)	218 (57.1)	33 (39.7)	5 (42)	4 (80)
Age, y. median (range)	70.1 (32-92)	68 (28-88)	65 (48-85)	62 (49-80)
Heavy chain, n (%):				
- Ig G	230 (60.2)	46 (55.4)	6 (50)	3 (60)
- Ig A	92 (24.1)	18 (21.7)	2 (17)	0
- light chain only	57 (14.9)	18 (21.7)	4 (33)	2 (40)
- IgD or IgM	3 (0.7)	1 (1.2)	0	0
Light chain, n (%):				
- kappa	247 (64.6)	54 (65.1)	5 (42)	2 (40)
- lambda	135 (35.3)	29 (34.9)	7 (58)	3 (60)
Haemoglobin, g/L. Median (range)	104 (56–171)	99 (68-139)	92 (65-144)	103 (40-129)
WBC. $x10^9$/L. Median (range)	6.2 (1.7–22.6)	5.9 (0.9-16.2)	6.3 (3.7-7.0)	24 (63-8)
Platelets. $x10^9$/L. Median (range)	214 (50–558)*	223 (58-493)	86 (24-174)*	138 (28-242)
Calcium, mg/dL. Median (range)	9.3 (7.2–14.8)	9.4 (7.3-14.2)	10.1 (8.6-15.8)	9.4 (9.0-11.4)
Creatinine, mg/dL. Median (range)	1.1 (0.3–11.9)	1.1 (0.5-12.2)	1.5 (0.6-8.0)	1.1 (0.6-6.8)
Lytic lesions. n (%):				
- None	172 (45.0)	39 (46.9)	7 (58)	3 (60)
- One to 3	186 (48.7)	43 (51.8)	4 (33)	1 (20)
- More than 3	24 (6.3)	1 (1.2)	1 (8)	1 (20)
D-S stage. n (%):				
- I	55 (14.4)	7 (8.4)	1 (8)	2 (40)
- II	156 (40.8)	41 (49.4)	3 (25)	0
- III	171 (44.7)	35 (42.2)	8 (67)	3 (60)
- B (creat >2mg/dL)	64 (16.7)	18 (21.7)	5 (42)	
β2-m, mg/L. median (range)	4.6 (1.0–48.0)	5.0 (1.7-35.1)	5.8 (2.9-31)	6.7 (4.9-7)
Albumin, g/L. median (range)	37.0 (15.0-48.9)	35.7 (20.0-43.0)	31 (24-45)	38.1 (29-47)
ISS stage. n (%):				
- I	85 (22.2)	13 (15.7)	1 (8)	0
- II	132 (34.6)	28 (33.7)	4 (33)	1 (20)
- III	137 (35.8)	36 (43.4)	7 (58)	3 (60)
- NA	28 (7.3)	6 (7.2)		1 (20)
LDH UNL, n (%)	59 (15.4)	11 (13.2)	4 (33)	2 (40)
Bone marrow PC, %. median (range)	36 (1-100)**	48 (2-100)	53 (38-90)**	43 (18-95)
Extramedullary disease, n (%)	77 (20.1)	11 (13.2)	3 (25)	1 (20)
Cytogenetics, n/assessed (%)				
- t(4;14).	7/164 (4.2)	5/31 (16.1)	0/4 (0)	0/3 (0)
- del 17p.	17/177 (9.6)	3/39 (7.7)	1/8 (12)	1/3 (33)

*p=0.001. **p=0.006. WBC: White blood cells. D-S: Durie-Salmon. β2-m: beta 2 microglobulin. ISS: International Staging System. LDH: Lactate dehydrogenase. UNL: Upper normal limit. PC: plasma cells.

Table 2. Risk factors for overall survival, overall cohort.

	Univariate*			Multivariate*		
	RR	95% CI	p	RR	95% CI	P
5-20% circulating PC	4.0	2.1 – 7.3	<.001	4.9	2.6 – 9.3	<.001
>65 years	2.1	1.6 – 2.8	<.001	2.0	1.5 – 2.8	<.001
D-S stage III	1.6	1.2 – 2.0	0.001	1.7	1.2 – 2.2	<.001
Creatinine >2mg/mL	1.7	1.3 – 2.3	0.001	1.5	1.1 – 2.1	0.010
ISS II or III	2.4	1.6 – 3.5	<.001	1.7	1.1 – 2.6	0.014
New drugs upfront	1.5	1.1 – 2.0	<.001	1.6	1.2 – 2.2	<.001
LDH UNL	1.3	0.9 – 1.9	0.07	1.4	0.9 – 1.9	0.094

RR: relative risk. CI: confidence interval. PC: plasma cells. D-S: Durie-Salmon. ISS: International staging System. LDH: lactate dehydrogenase. UNL: Upper normal limit.
* Cox model.

Table 3. Factors associated with overall survival in patients treated with novel drugs upfront.

	Univariate*			Multivariate*		
	RR	95% CI	p	RR	95% CI	P
≥5% circulating PC	4.8	2.5 – 9.1	<.001	4.5	2.4 – 8.8	<.001
>65 years	1.4	0.9 – 2.1	0.060	1.3	0.8 – 1.9	0.186
D-S stage III	1.3	0.9 – 1.9	0.102	1.3	0.9 – 2.0	0.132
Creatinine >2mg/mL	1.8	1.2 – 2.8	0.007	1.5	0.9 – 2.4	0.082
ISS II or III	1.9	1.1 – 3.3	0.010	1.6	0.9 – 2.7	0.092

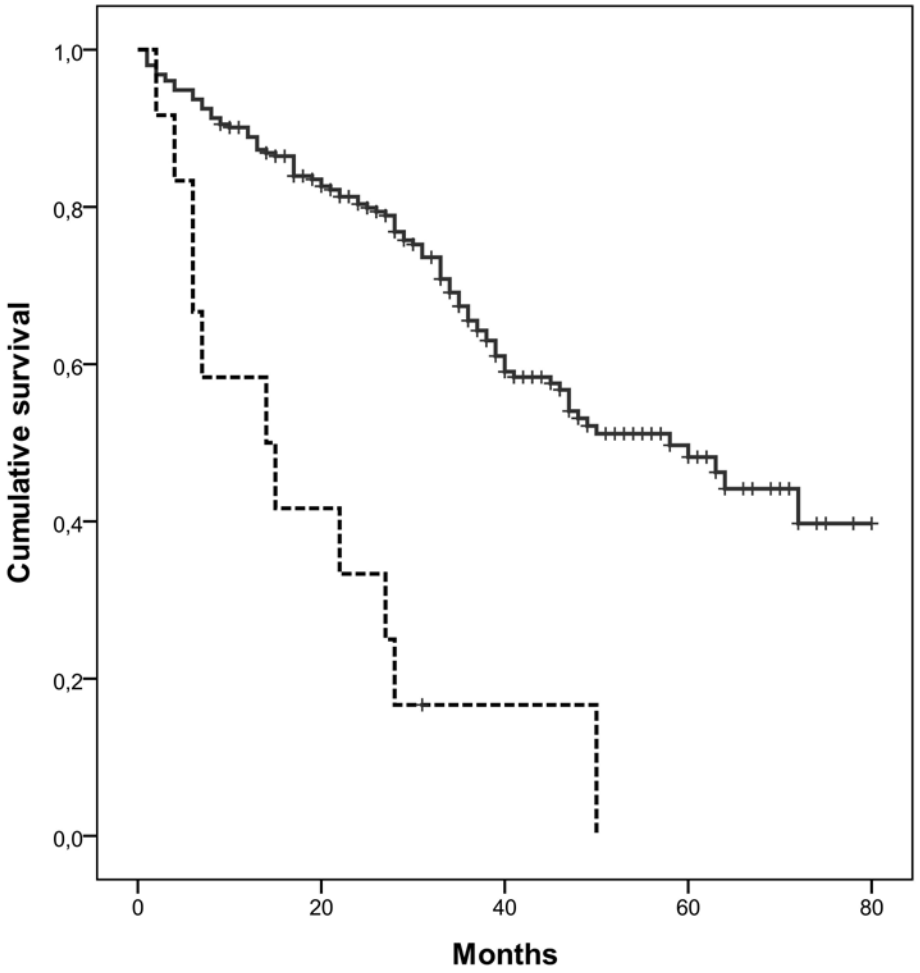
RR: relative risk. CI: confidence interval. PC: plasma cells. D-S: Durie-Salmon. ISS: International staging System. LDH: lactate dehydrogenase.* Cox model.

FIGURE LEGENDS

Figure 1. Overall survival according to the circulating plasma cell (PC) group in patients with multiple myeloma ($p<0.001$)

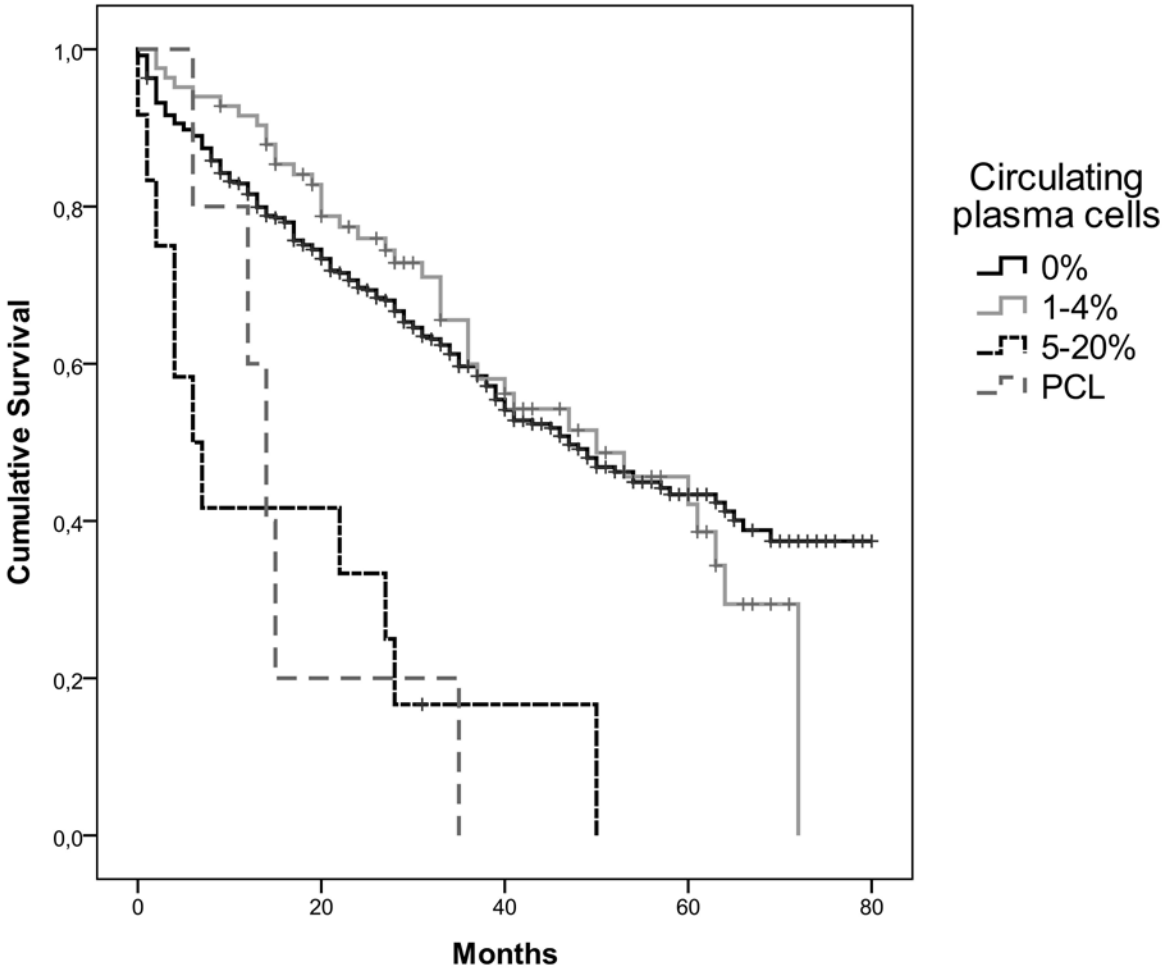
Figure 2. Overall survival according to the circulating plasma cells (PC) in patients with multiple myeloma and plasma cell leukemia (PCL) treated with novel drugs upfront ($p<0.001$)

Figure 1



<5% PC	253	191	92	33	2
≥5% PC	12	5	1	0	0

Figure 2



0% PC	382	253	127	50	2
1-4% PC	83	62	31	13	0
5-20% PC	12	5	1	0	0
PCL	5	1	0	0	0

Supplementary material

Table Supplementary 1. Clinical characteristics of patients with less than 5% versus those with ≥5% circulating plasma cells in peripheral blood.

Circulating plasma cells	<5%	≥5%
N (% overall)	465 (96.5)	17 (3.5)
Male, n (%)	251 (53.9)	9 (53)
Age, y. median (range)	70 (32-92)	64 (48-85)
Heavy chain, n (%):		
- IgG	276 (59.4)	9 (53)
- IgA	110 (23.7)	2 (12)
- light chain only	75 (16.1)	6 (35)
- IgD or IgM	4 (0.8)	0
Light chain, n (%):		
- kappa	301 (64.7)	7 (41)
- lambda	164 (35.3)	10 (59)
Haemoglobin, g/L. Median (range)	103 (56–171)	95 (40-144)
WBC x10^9/L. Median (range)	6.2 (0.9–22.6)	6.9 (3.7-63)
Platelets x10^9/L. Median (range)	214 (50–558)*	91 (24-242)*
Calcium, mg/dL. Median (range)	9.4 (7.2–14.8)	9.9 (9.0-15.8)
Creatinine, mg/dL. Median (range)	1.1 (0.3–12.2)	1.4 (0.6-8.0)
Lytic lesions. n (%):		
- None	211 (45.4)	10 (59)
- One to 3	229 (49.2)	5 (29)
- More than 3	25 (5.4)	2 (12)
D-S stage. n (%):		
- I	62 (13.3)	3 (17.6)
- II	197 (42.4)	3 (17.6)
- III	206 (44.3)	11 (64.7)
- B (creat >2mg/dL)	82 (17.6)	7(41)
β2-m, mg/L. median (range)	4.7 (1.0–48.0)	5.7 (2.9-31)
Albumin, g/L. median (range)	36.2 (15.0-48.9)	32 (24-45)
ISS stage. n (%):		
- I	98 (21.1)	1 (6)
- II	160 (34.4)	5 (29)
- III	173 (37.2)	10 (59)
- NA	34 (7.3)	1 (6)
LDH UNL, n (%)	70 (15.1)*	6 (35)*
Bone marrow PC, %. median (range)	39.5 (1-100)*	51 (18-95)*
Extramedullary disease, n (%)	88 (18.9)	4 (23.5)

*p<0.001. WBC: White blood cells. D-S: Durie-Salmon. β2-m: beta-2 microglobulin. ISS: International Staging System. LDH: Lactate dehydrogenase. UNL: Upper normal limit. PC: plasma cells.