Flow-cytometry-identified plasma cell and plasma-cell-neighboring components are associated with disease burden, prognosis and survival in multiple myeloma patients

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Background & Objective

- The microenvironment influences multiple myeloma (MM) persistence and progression.
- Here we examined various indices in patients and studied their association with disease burden, prognosis and survival.

Methods

- We retrospectively collected relevant hematological and biochemistry indices and flow-cytometry data of bone-marrow (BM) aspirates from 58 patients (F:M [%] 53.4: 46.6, age [mean ± SD, range] 66.8 ± 11.63 years, 39-85) at their diagnosis.
- Correlations between determinants and incidences of clonal plasma cells (PCs), international staging system (ISS), progression free survival (PFS) and overall survival (OS) were performed using the JMP software.

Results

Patients Characteristics

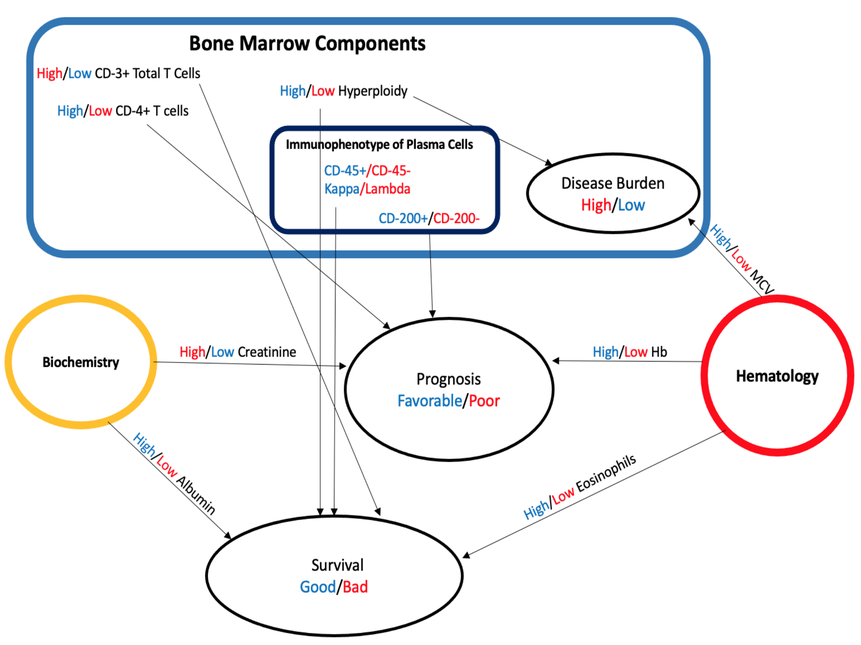
Age [years]	Average	66.86
	Standard Deviation	11.63
	Median [range]	68 [39-85]
Sex	Male	27 (47%)
	Female	31 (53%)
Progression Free Survival (PFS) [months]	Size of Population	48
	Average	33.8
	Standard Deviation	31
	Median [range]	30 [1-122.9]
Overall Survival (OS) [months]	Size of Population	47
	Average	41.9
	Standard Deviation	35.58
	Median [range]	35.4 [1-124.5]
Prognostic Groups		
International Staging System (ISS)	Size of Population	56
	Number of Patients with ISS-1	26 (46.4%)
	Number of Patients with ISS-2	9 (16%)
	Number of Patients with ISS-3	21 (37.5%)
Revised International Staging System (R-ISS)	Size of Population	25
	Number of Patients with R-ISS-1	9 (36%)
	Number of Patients with R-ISS-2	15 (60%)
	Number of Patients with R-ISS-3	1 (4%)

FC Indices of Immune Cells in the Myeloma Microenvironment

	Sample Size (n=)	Average	Standard Deviation	Median [range]	Notes
Total CD45+	30	64.57	19.08	[21.12-93.44] 63.8	
Lymphocytes CD45+/SSC low	30	15.26	11.13	[3.57-24.7] 14.85	
Monocytes CD45+/CD64 high/SSC low	30	4.3	2.59	[0.75-12.5] 4.15	
Mature Granulocytes	30	29.75	15.37	[4.7-68.1] 31.41	
Immature Granulocytes	30	12.45	10.55	[0.7-42.2] 10.05	
CD3+ (Total T-Cells)	30	72.19	12.26	[46.2-94.8] 73.2	
CD4+ T-Cells	30	45.24	15.93	[2.3-68.7] 45.5	
CD8+ T-Cells	30	49.72	1.85	[26.8-81.4] 50.75	
CD4+/CD8+ ratio	30	1.03	0.58	[0.08-2.5] 0.87	
DP T-Cells	30	1.78	2.66	[0.1-14.1] 0.85	
DN T-Cells	30	4.74	3.3	[1.4-15.4] 4.05	
CD3-/CD56+ (NK Cells)	30	10.8	7.92	[1.5-38.5] 8.5	
NKT Cells CD3+/CD56+	30	10.61	6.03	[1.7-26.4] 9.65	
FC Indices of PCs					
% PCs in FC	58	10.67	18.14	[0.4-84.5] 4.26	
% Of Proliferating PCs	40	11.06	8.1	[0.96-32.47] 9.75	
Pleuidy	40	-	-	-	80% Diploid (n=32); 20% Hyperploid (n=8)
	Sample Size (n=)	**Positive**	**Negative**		
CD138	58	-	-	-	
CD38	58	55 (95%)	3 (5%)	-	
CD56	57	(66.7%) 38	(33.3%) 19	-	
CD19	51	(8%) 4	(92%) 47	-	
CD45	45	(33%) 15	(67%) 30	-	
CD200	27	(78%) 21	(22%) 6	-	
CD27	30	(53%) 16	(47%) 14	-	
CD81	26	(23%) 6	(77%) 20	-	
K/L Restriction	55				58% Kappa (n=32) 42% Lambda (n=23)

Illustration :

The possible relationship between laboratory findings and disease severity in patients with multiple myeloma



Correlations between hematological and biochemical indices and the percentage of clonal PCs, ISS, PFS and OS

	Abs. Basophils [cells/µL]	Abs. Eosinophils [cells/µL]	Abs. Lymphocytes [cells/µL]	Abs. Monocytes [cells/µL]	Abs. Neutrophils [cells/µL]	Platelets [plts/µL]	MCV [fL]	Hb [g/dL]	WBCs [cells/µL]
% Of PCs in Biopsy	r = 0.14 p-value = 0.3	r = 0.08 p-value = 0.58	r = 0.37 p-value = 0.07	r = 0.07 p-value = 0.62	r = -0.22 p-value = 0.12	r = 0.08 p-value = 0.58	r = -0.32 p-value = 0.02	r =0.03 p-value = 0.83	r = 0.1 p-value = 0.45
PFS (months)	r = 0.17 p-value=0.27	r = 0.3 p-value=0.05	r = 0.28 p-value = 0.06	r = 0.14 p-value=0.36	r = 0.09 p-value=0.85	r = 0.14 p-value=0.3	r = 0.04 p-value = 0.75	r = 0.09 p-value=0.54	r = 0.14 p-value = 0.76
OS (months)	r = 0.26 p-value=0.07	r = 0.04 p-value=0.77	r = 0.11 p-value=0.47	r = 0.22 p-value=0.14	r = 0.17 p-value=0.28	r = 0.14 p-value=0.36	r = 0.03 p-value=0.78	r = 0.03 p-value=0.83	r = 0.17 p-value=0.27
ISS-1 vs. ISS-2/3	0.03±0.02 vs. 0.03±0.03 p-value = 0.66	0.08±0.07 vs. 0.15±0.19 p-value = 0.2	2.02±0.68 vs. 1.8±0.82 p-value = 0.34	0.596±0.27 vs. 0.59±0.34 p-value = 0.98	5.22±2.5 vs. 4.5±3.18 p-value = 0.38	237.86±102.1 vs. 198.6±89.01 p-value = 0.15	85.58±12.45 vs. 82.67±21.54 p-value = 0.57	12.0±1.9 vs. 9.84±1.76 p-value=0.0002	7.95±2.78 vs. 7.12± 3.4 p-value=0.35

	B2M [mcg/mL]	LDH [U/L]	Total Protein [g/dL]	Albumin [g/dL]	Ca2+ [mg/dL]	Cr [mg/dL]
% Of PCs in Biopsy	r = 0.09 p-value = 0.53	r = -0.2 p-value = 0.12 * after exclusion of a single abnormal value r = -0.3 p-value = 0.03	r = 0.3 p-value = 0.03	r = 0.1 p-value = 0.4	r = 0.07 p-value = 0.6	r = -0.24 p-value = 0.1
PFS (months)	r = -0.28 p-value = 0.05	r = 0.12 p-value = 0.4	r = 0.17 p-value = 0.74	r = 0.36 p-value = 0.01	r = 0.35, p-value = 0.03	r = -0.36, p-value = 0.02
OS (months)	r = 0.25 p-value = 0.1	r = 0.06 p-value = 0.67	r = 0.03 p-value = 0.8	r = 0.33 p-value = 0.02	r = 0.3 p-value = 0.07	r = 0.24, p-value = 0.14
ISS-1 vs. ISS-2/3	2.72±0.75 vs. 8.35±5.35 p-value < 0.001	220.64±125.38 vs. 188.7±58.9 p-value = 0.22	7.6 ± 1.5 vs. 7.16 ± 1.7 p-value = 0.38	4.01±0.32 vs 3.22±0.58 p-value < 0.001	9.46±0.58 vs. 9.13±1.05 p-value = 0.23	0.77±0.15 vs. 2.25±1.84 p-value = 0.0016

Correlations between flow cytometry indices and the percentage of clonal PCs, ISS, PFS and OS

	Kappa vs. Lambda Restriction	CD81 (-) vs. (+)	CD27 (-) vs. (+)	CD200 (-) vs. (+)	CD45 (-) vs. (+)	CD19 (-) vs. (+)	CD56 (-) vs. (+)	CD38 (-) vs. (+)	CD 138	Diploid vs. Hyperdiploid	% Of Proliferating Cells	% PCs in FC
% Of PCs in Biopsy	37.26%±26.96 vs. 42.98±31.78 p-value = 0.32	37.47±28.53 vs. 43.57±41.9 p-value = 0.67	49.28±32.87 vs. 35.56±28.26 p-value = 0.22	63.33±31.57 vs. 33.73±29.59 p-value = 0.04	42.13 %±31.56 vs. 31.77±23.84 p-value = 0.29	41.76%±29.37 vs. 13.67±15.17 p-value = 0.1	40.5% ±31.43 vs. 38.55±28.33 p-value = 0.82	10%±10 vs. 40.86%±28.7 p-value = 0.07	100 %	35.64%±30.92 vs. 43.75%±23.86 p-value = 0.49	r = 0.04 p-value=0.77	r = 0.19 p-value = 0.16
PFS (months)	41.83±31.11 vs. 23.17±22.42 p-value=0.028	30.53±31.87 vs. 14.48±13.66 p-value = 0.34	35.92±36.24 vs. 23.38±23.54 p-value = 0.29	26.55±6.64 vs. 26.91±33.3 p-value = 0.98	23.2±19.83 vs. 53.14±43.37 p-value = 0.006	32.58 ±29.02 vs. 23.15±27.6 p-value = 0.65	40.55±36.99 vs. 27.39±22.33 p-value = 0.13	51.95±22.9 vs. 32.56 ±31.38 p-value = 0.3	33.77 ±31.08	26.43±22.19 vs. 42.5±42.61 p-value = 0.18	0 p-value = 0.96	0 p-value = 0.98
OS (months)	51.28±36.34 vs. 30.37±27.33 p-value = 0.04	32.85±35.46 vs. 23.27±15.88 p-value = 0.6	39.41±37.69 vs. 30.71±35.38 p-value = 0.57	30.99±5.13 vs. 31.14±36.26 p-value = 0.99	28.213 ±22.39 vs. 65.35±47.82 p-value = 0.003	40.66±33.85 vs. 34.22±43.25 p-value = 0.79	50.58±38.02 vs. 35.06±31.02 p-value = 0.14	73.82±30.71 vs. 40.46±35.41 p-value = 0.19	41.88 ±35.58	32.38±29.5 vs. 63.33±38.35 p-value = 0.03	r = 0.33 p-value 0.06	r = 0.08 ,p-value = 0.57
ISS-1 vs. ISS-2/3	ISS-1 61.5% vs. 38.5% ISS-2/3 53.5% vs. 46.5% p-value = 0.55	ISS-1 70% vs. 30% ISS-2/3 83% vs. 17% p-value = 0.41	ISS-1 46.15% vs. 53.85% ISS-2/3 50% vs. 50% p-value = 0.84	ISS-1 7.7% vs. 92.3% ISS-2/3 38.5% vs. 61.5% p-value = 0.054	ISS-1 65% vs. 35% ISS-2/3 74% vs. 26 p-value = 0.52	ISS-1 92% vs. 8% (+) 100% vs. 0% p-value = 0.1	ISS-1 38.46 vs. 31.5 ISS-2/3 27.6% vs. 72.4% p-value 0.39	ISS-1 7.7% vs. 92.3% ISS-2/3.93.3 vs. 6.7% p-value = 0.47		ISS-1 73.7% vs. 26.32% ISS-2/3 84.2% vs. 15.8% p-value = 0.42	11.2±7.28 vs. 10.89±9.4 p-value = 0.91	13.6±22.58 vs. 8.69±13.79 p-value = 0.32

	NKT Cells CD3+/CD56+	NK Cells CD3-/CD56+	DN T-Cells	DP T-Cells	CD4+/CD8+ ratio	CD8+ T Cells	CD4+ T Cells	CD3+ Total T Cells	iGr	mGr	Monocytes CD45+/CD64 high/SSC low	Lymphocytes CD45+/SSC low	Total CD45+
% Of PCs in Biopsy	r = 0.21 p-value = 0.23	r = 0.07 p-value = 0.67	r = 0.04 p-value = 0.78	r = 0.17 p-value = 0.35	r = 0.26 p-value = 0.15	r = 0.3, p-value = 0.08	r = 0.2 p-value = 0.26	r = 0.17 p-value = 0.3	r = 0.05 p-value = 0.74	r = 0.14 p-value = 0.38	r = 0.22 p-value = 0.178	r = 0.3 p-value = 0.07	r = 0.17 p-value = 0.3
PFS (months)	r = 0.32, p-value = 0.12	r = 0.14, p-value = 0.44	r = 0.04, p-value = 0.8	r = 0.28, p-value = 0.158	r = 0.14, p-value = 0.49	r = 0.2, p-value = 0.32	r = 0.13, p-value = 0.54	r = 0.24, p-value = 0.23	r = 0.01, p-value = 0.57	r = 0.11, p-value=0.57	r = 0.19, p-value=0.35	r = 0.26, p-value =0.2	r = 0.03, p-value = 0.87
OS (months)	r = 0.22, p-value = 0.27	r = 0.33, p-value = 0.1	r = 0.2, p-value = 0.36	r = 0.26, p-value = 0.2	r = 0.28, p-value = 0.176	r = 0.1, p-value = 0.56	r = 0.3, p-value = 0.15	r = -0.41, p-value = 0.04	r = 0.17, p-value = 0.43	r = 0.24, p-value = 0.25	r = 0.17, p-value = 0.43	r = 0.28, p-value = 0.79	r = 0.09, p-value = 0.65
ISS-1 vs. ISS-2/3	9.78±3.58 vs. 11.83±7.72 p-value=0.38	8.38±4.3 vs. 12.98±10.06 p-value=0.14	4.63±2.78 vs. 4.56±3.95 p-value =0.95	1.67±1.53 vs. 1.16±1.1 p-value =0.32	1.14±0.43 vs. 0.81±0.57 p-value =0.1	46.84 ±9.56 vs. 53.95 ±15.93 p-value =0.17	49.52± 9.65 vs. 38.5±17.5 p-value= 0.05	72.6±9.83 vs. 70.83±14.66 p-value= 0.71	12.48±11.25 vs. 11.03±9.7 p-value=0.72	30.07 ±16.12 vs. 29.82±14.72 p-value=0.97	3.39±1.4 vs. 5±3.3 p-value=0.11	13.64±5.4 vs. 16.28±15.04 p-value=0.55	69.52±19.95 vs. 58.56±17.98 p-value=0.14

Red – statistically significant correlation (p≤0.05) ; Green – possible correlation, not clinically significant (0.05<p<0.1)

Conclusions

These preliminary data raise suggest the clinical relevance of a combination of PC and PC-neighboring flow-cytometry-identified biomarkers as indicators of disease burden, prognosis and survival in MM.