Article

Pretreatment serum levels of IL-1 receptor antagonist and IL-4 are predictors of overall survival in multiple myeloma patients treated with bortezomib

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Abstract: Multiple myeloma (MM) is characterized by the malignant proliferation of monoclonal plasma cells in the bone marrow with an elevation in monoclonal paraprotein, renal impairment, hypercalcemia, lytic bony lesions, and anemia. Immune cells and associated cytokines play a significant role in MM growth, progression, and dissemination. While some cytokines and their clinical significance are well described in MM biology, others remain relatively unknown. The aim of the present study was to assess the impact of pretreatment serum levels of 27 selected cytokines on progression-free survival (PFS) and overall survival (OS) in MM patients before first-line therapy with bortezomib-based regimens. Serum cytokine levels were assayed with a Bio-Rad Bio-Plex Pro Human Cytokine 27-Plex Assay on the MAGPIX Multiplex Reader and the Bio-Plex® 200 System (Bio-Rad) including IL-1β, IL-1Ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, Eotaxin, FGF, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF-BB, RANTES, TNF-α, and VEGF. A total of 61 MM patients were examined. Most patients received a bortezomib, cyclophosphamide and dexamethasone (VCD) chemotherapy regimen. In the final multivariate model, IL-13 cytokine level (HR 0.1411, 95% CI: 0.0240-0.8291, p = 0.0302), and ASCT (HR 0.3722, 95% CI: 0.1826-0.7585, p=0.0065) significantly impacted PFS. Furthermore, ASCT (HR 0.142, 95% CI: 0.046-0.438, p=0.0007), presence of bone disease at diagnosis (HR 3.826, 95% CI: 1.471-9.949, p=0.0059) and two cytokine levels- IL-1Ra (HR 1.017, 95% CI: 1.004-1.030, p= 0.0091) and IL-4 (HR 0.161, 95% CI: 0.037-0.698, p = 0.0147) were independent predictors of OS. Three clusters of MM patients were identified with different cytokine profiles. In conclusion, serum pretreatment levels of IL-13 and IL-4 are predictors of better PFS and OS, respectively, whereas IL-1Ra pretreatment levels negatively impact OS in MM patients treated with bortezomib-based chemotherapy. Cytokine signature profile may have a potential influence on the outcome of patients treated with bortezomib.

1. Introduction

Multiple myeloma (MM) is a neoplasm characterized by aberrant expansion of monoclonal plasma cells with bone marrow involvement, plasma renal impairment, hypercalcemia, lytic bony lesions, and anemia [1,2]. It is the second-most prevalent blood cancer in the United States and Europe. According to the WHO, 176 404 new MM cases and 117 077 deaths due to MM occurred worldwide in 2020 [3]. Multiple myeloma usually happens around the age of 60 and is more common in men than women [4,5].

Multiple myeloma is an incurable disease characterized by substantial morbidity and mortality [5]. However, survival of patients with MM has improved significantly over the past 15 years following the introduction of several novel therapeutic agents, including proteasome inhibitors (PI), immunomodulating agents (IMiD) and monoclonal antibodies [5,6]. In addition, high-dose chemotherapy with autologous stem-cell transplantation (ASCT) significantly prolongs overall survival (OS) in younger patients. The MM treatment options have never been so broad, and new biomarkers are urgently needed to identify an adequate treatment regimen for a particular patient.

A crucial role in the treatment of MM is played by proteasome inhibitors, of which bortezomib was the first-in-class selective and reversible example. It was approved for medical use in the United States in 2003 and in the European Union in 2004 [7]. Bortezomib demonstrates antiproliferative and antitumor activity, and its use has been a breakthrough in treating MM [8]. However, most patients treated with bortezomib-based regimens eventually relapse and often demonstrate multiple drug resistance [9].

Immune cells and associated cytokines play a crucial role in the growth, progression, and dissemination of MM. While the roles of some cytokines, such as IL-6, TGF-β, and IL-1β, are well described in the MM biology, those of others remain unclear [10-13]. Previous reports indicated that bortezomib treatment is associated with significantly reduced serum levels of many cytokines and chemokines, including IL-1α/β, IL-3, IL-4, IL-5, IL-6, IL-8, IL-12p40, IL-12p70, IFN-γ, TNF-α, MIP-1α/β, GM-CSF, CXCL1, RANTES, VEGF, Eotaxin, and IL-13 [11-14]. Our previous study investigated circulating cytokine, growth factor, and chemokine levels and their association with selected clinical and laboratory disease characteristics [11]. Several cytokines were found to be involved in the pathogenesis of MM and some were useful in predicting treatment response in bortezomib-treated patients; in particular, higher levels of MIP-1α and lower levels of MIP-1β and IL-9 were associated with better response to treatment, while higher IL-1Ra and IL-8 levels were associated with osteolytic bone symptoms.

The aim of the present study was to assess the impact of pretreatment serum levels of 27 selected cytokines on progression-free survival (PFS) and overall survival (OS) in MM patients treated with bortezomib-based regimens as first-line therapy.

2. Materials and Methods

2.1. Patients

The study group comprised 61 patients with MM treated at the Department of Hematology, Copernicus Memorial Hospital, Lodz, Poland, from February 2016 to September 2019. All of the patients received bortezomib-based treatment as first-line therapy. Response to treatment and progression events were classified according to the International Myeloma Working Group [14,15]. The study was conducted according to good clinical and laboratory practice rules and the principles of the Declaration of Helsinki. All procedures were approved by the local ethical committee (The Ethical Committee of the Medical University of Lodz, No. RNN/103/16/KE).

2.2. Cytokine Analysis

The method of cytokine analysis has previously been described in detail [12]. Brifly, patients with preexisting conditions (allergy/infections) that may cause changes in the cytokine profile were excluded before blood sample collection. After the collection of whole blood, it was left undisturbed at room temperature for 30 minutes. The samples were then centrifuged at 2000 x g for 10 minutes. Serum samples from all studied patients were stored at -80° C.

Serum cytokine levels were assayed with Bio-Rad Bio-Plex Pro Human Cytokine 27-Plex Assay on the MAGPIX Multiplex Reader and the Bio-Plex® 200 System (Bio-Rad) as described previously [12]. Briefly, the Luminex® xMAP® technology based on immunoassay methods is capable of simultaneously quantifying 27 targets: IL-1β, IL-1 receptor antagonist (IL-1Ra), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, Eotaxin (CCL11), fibroblast growth factor (FGF), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN-γ), IFN-γ-induced protein 10 (IP-10), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein- (MIP) 1α and MIP-1β, platelet-derived growth factor BB (PDGF-BB), RANTES (Regulated on Activation, Normal T Expressed and Secreted, CCL5), tumor necrosis factor α (TNF-α), and vascular endothelial growth factor (VEGF).

The concentration of each cytokine was extrapolated from the calibration curve (individual for each cytokine) determined independently for each experiment (each plate). All samples were analyzed in duplicate.

2.3. Statistical analysis

Nominal variables were expressed as percentages and analyzed using the Chi-square test with appropriate corrections if needed: the Yates correction for continuity or Fisher's exact test. The normality of the distribution of continuous variables was verified with the Shapiro-Wilk test. Continuous variables were presented as mean ± standard deviation (SD). ANOVA with post hoc Tukey's test was used to compare cytokine concentrations between identified MM patient clusters. Heatmap and hierarchical agglomerative clustering were used to evaluate clusters of patients with similar plasma cytokine profiles.

Survival analysis was conducted using a Kaplan–Meier estimate with univariate and multivariate Cox's proportional hazards models, as well as the log-rank test. Optimal cutoff values were determined using Cutoff Finder [16]. In Cox's models, cytokine concentrations were divided by 10 to interpret coefficient values more straightforward. Analyses were conducted using Statistica 13.1 (TIBCO, Palo Alto, CA, USA). P values < 0.05 were considered statistically significant.

3. Results

3.1. Patients' Characteristics

The demographic, clinical, and laboratory characteristics of the MM patients enrolled in the study are presented in Table 1. The group comprised 32 men and 29 women with a mean age of 61.9 ± 11.3 years (range: 38.3-83.7). The distribution according to the ISS stage I-III was 27.9%, 21.3% and 47.5%, respectively (missing data- 3.3%). The predominant isotype of monoclonal protein was IgG kappa (52.5%). Regarding CRAB symptoms, most patients (59%) presented with bone disease, 34.4% displayed Hb <10 g/dL, hypercalcemia was noted in 19.7%, and renal failure was observed in 16.4% of patients. Cytogenetics data were available for 33 patients (54.1%); the most common abnormalities were amp(1q) followed by del(13q), with frequencies of 18 (54.5%) and 8 (24.5%), respectively. The majority of patients (80.3%) received a bortezomib, cyclophosphamide, and dexamethasone (VCD) chemotherapy regimen. The objective response rate with primary induction treatment for all patients was 83.6%, including 60.6% ≥ VGPR. Complete response was achieved by 24 (39.3%) of patients. More than one third (37.7%) of patients underwent ASCT.

Table 1. The characteristics of patients treated with bortezomib-based therapy. Data are presented as frequency and percentage (%) unless otherwise specified.

Characteristics	Total
Number of patients	61 (100)
Gender	M: 32 (52.5)
	F: 29 (47.5)
Age at diagnosis	61.9 ± 11.3
mean + SD (range)	(38.3-83.7)
Bortezomib regimen:	
VCD	49 (80.3)
VMP	5 (8.2)
VTD	4 (6.6)
Other	3 (4.9)
Paraprotein	
IgG	33 (54)
IgA	14 (23)
LCD	14 (23)
Bone disease at diagnosis	36 (59)
Calcium > 2.75 mmol/l at diagnosis	12 (19.7)
HB < 10g/dL at diagnosis	21 (34.4)
Creatinine > 2 mg/dL at diagnosis	10 (16.4)
International Staging System (ISS)	I -17 (27.9)
	II-13 (21.3)
	III-29 (47.5)
Beta2-microglobuline increased (> 3mg/L)	40 (65.6)
LDH >240U/L	7 (11.5)
Cytogenetics*	N=33
t(11;14)	1 (3)
t(4;14)	5 (15.2)
t(14;16)	0
t(14;20)	0
del(17p)	3 (9.1)
amp(1q)	18 (54.5)
del(13q)	8 (24.2)

*Cytogenetics data were available for 33 patients (54.1%). Abbreviations: LCD—light chain disease; VCD—bortezomib, cyclophosphamide, and dexamethasone; VD—bortezomib and dexamethasone; VMP—bortezomib, melphalan, and prednisone; VTD—bortezomib, thalidomide, and dexamethasone.

3.2. Prognostic impact of clinical variables

Data on PFS and on OS was available for 61 patients. The median PFS and overall survival OS of the cohort were 13.1 and 51.8 months, respectively. Univariate Cox regression analyses of clinical variables for PFS and OS are summarized in Table 2. Only autologous stem cell transplantation (ASCT) during the treatment schedule was clinical

factor influencing both PFS (HR 0.33, 95% CI: 0.17 – 0.64, p = 0.001) and OS (HR 0.23, 95% CI: 0.09 – 0.62, p = 0.004). The corresponding Kaplan-Meier plots are shown in Figure 1.

Table 2. Univariate Cox regression analyses of basic clinical variables for progression-free survival and overall survival.

| Variable	PFS					OS				
	Coefficient	*p*	HR	95% CI		Coefficient	*p*	HR	95% CI	
				Lower	Upper				Lower	Upper
ISS III	-0.13	0.680	0.88	0.48	1.60	0.78	0.060	2.18	0.97	4.90
ASCT	**-1.11**	**0.001**	**0.33**	**0.17**	**0.64**	**-1.46**	**0.004**	**0.23**	**0.09**	**0.62**
HB < 10 g/dL at diagnosis	0.12	0.720	1.13	0.59	2.17	0.12	0.778	1.13	0.49	2.58
Calcium > 2.75 mmol/l at diagnosis	0.46	0.213	1.58	0.77	3.23	-0.40	0.464	0.67	0.23	1.95
Creatinine > 2 mg/dL at diagnosis	-0.48	0.323	0.62	0.24	1.60	0.07	0.893	1.08	0.36	3.18
Bone disease	0.64	0.054	1.89	0.99	3.61	0.61	0.156	1.85	0.79	4.30
Age >70	0.34	0.323	1.41	0.71	2.80	0.63	0.118	1.87	0.85	4.10
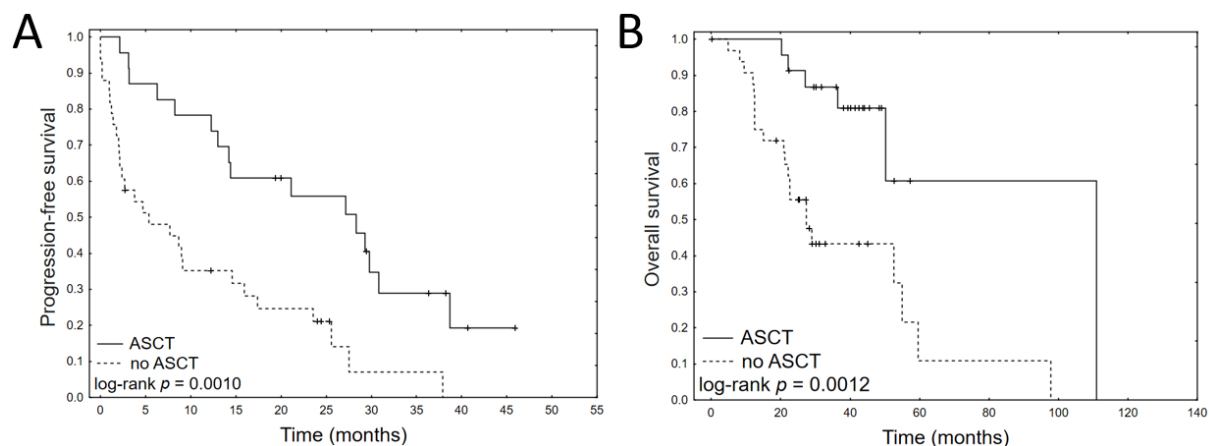
Figure 1. Kaplan–Meier plots of progression-free survival (A) and overall survival (B) for ASCT in MM patients treated with bortezomib.

3.3. Prognostic impact of cytokine levels

Univariate Cox regression analyses of pretreatment serum cytokine levels for PFS and OS are provided in Table 3. Only IL-13 serum level significantly impacted PFS (HR 0.1398, 95% CI: 0.0272– 0.7189, p = 0.0185). Regarding OS, five cytokines were significant in univariate analyses: IL-13 (HR 0.0121, 95% CI: 0.0004– 0.3271, p = 0.0087), IL-1ra (HR 1.0527, CI:1.0177–1.0889, p = 0.0029), IL-4 (HR 0.1899, CI: 0.0401–0.9000, p = 0.0364), IL-7 (HR 0.6313, CI:0.3988–0.9993, p = 0.0497) and PDGF-BB (HR 0.9963, CI: 0.9930–0.9997 p = 0.0316). The Kaplan-Meier plots for dichotomized cytokine levels are provided in Figure 2.

The cytokines found to be significant in univariate analysis were entered into a multivariate model building with a stepwise backward Akaike information criterion (AIC) elimination procedure, together with well-established prognostic factors (ISS III, ASCT, age >70, and presence of bone disease).   Briefly, an optimized model is identified by creating multiple models, initially including all selected variables. The variable with the highest p-value is eliminated at each step, and an AIC value is calculated for the new model; this process is repeated until no variables remain. The final multivariate models

for PFS and OS with the lowest overall AICs are given in Table 4. The analysis confirmed that cytokine levels retained their importance in the simultaneous context of the clinical prognostic factors. The final model for PFS consisted of two variables: IL-13 cytokine level (HR 0.141, 95% CI: 0.024-0.829, p = 0.0302), and ASCT (HR 0.3722, 95% CI: 0.1826-0.7585, p = 0.0065). The final model for OS included ASCT (HR 0.142, 95% CI: 0.046-0.438, p = 0.0007), presence of bone disease at diagnosis (HR 3.826, 95% CI: 1.471-9.949, p = 0.0059) and two cytokine levels- IL-1Ra (HR 1.017, 95% CI: 1.0004-1.030, p = 0.0091) and IL-4 (HR 0.161, 95% CI: 0.037-0.698, p = 0.0147).

Table 3. Univariate Cox regression analyses of cytokine levels for progression-free survival and overall survival. Cytokine concentrations (pg/ml) were divided by 10 to allow a more straightforward interpretation of coefficient values.

| Cytokine	PFS					OS				
	Coefficient	p	HR	95% CI		Coefficient	p	HR	95% CI	
				Lower	Upper				Lower	Upper
Eotaxin	0.0022	0.9558	1.0022	0.9279	1.0824	-0.0954	0.0620	0.9090	0.8224	1.0048
FGF basic	-0.0825	0.6462	0.9208	0.6475	1.3096	-0.2964	0.2059	0.7435	0.4697	1.1769
G-CSF	-0.0009	0.8658	0.9991	0.9882	1.0101	0.0002	0.9755	1.0002	0.9852	1.0155
GM-CSF	-1.7105	0.0694	0.1808	0.0285	1.1456	-4,9713	0.0643	0.0069	0.00004	1.3441
IFN-γ	-0.0230	0.8837	0.9773	0.7184	1.3296	-0.1371	0.5175	0.8719	0.5756	1.3206
IL-10	-0.0005	0.9984	0.9995	0.6270	1.5934	0.0049	0.0531	1.0049	0.9999	1.0099
IL-12 (p70)	0.6213	0.3417	1.8613	0.5172	6.6991	0.3990	0.7549	1.4903	0.1217	18.2445
IL-13	**-1.9675**	**0.0185**	**0.1398**	**0.0272**	**0.7189**	**-4.4187**	**0.0087**	**0.0121**	**0.0004**	**0.3271**
IL-15	0.0138	0.5837	1.0139	0.9652	1.0650	-0.0110	0.7869	0.9890	0.9131	1.0713
IL-17	-0.0975	0.5954	0.9071	0.6329	1.3001	-0.4021	0.1243	0.6689	0.4006	1.1170
IL-1ra	0.0012	0.8111	1.0012	0.9917	1.0107	**0.0514**	**0.0029**	**1.0527**	**1.0177**	**1.0889**
IL-1β	-1.6824	0.1799	0.1859	0.0159	2.1737	-1.3066	0.5299	0.2707	0.0046	15.9668
IL-2	-0.3222	0.6034	0.7246	0.2149	2.4429	-1.2680	0.1562	0.2814	0.0488	1.6234
IL-4	-0.1722	0.7859	0.8418	0.2430	2.9165	**-1.6615**	**0.0364**	**0.1899**	**0.0401**	**0.9000**
IL-5	0.1369	0.2194	1.1467	0.9217	1.4268	-0.0654	0.6848	0.9367	0.6829	1.2847
IL-6	0.0328	0.9321	1.0334	0.4860	2.1970	-0.2548	0.6893	0.7751	0.2223	2.7027
IL-7	0.0450	0.7981	1.0460	0.7411	1.4762	**-0.4600**	**0.0497**	**0.6313**	**0.3988**	**0.9993**
IL-8	0.1698	0.3739	1.1850	0.8151	1.7229	0.0972	0.7127	1.1021	0.6570	1.8487
IL-9	0.0217	0.1296	1.0219	0.9937	1.0509	-0.0149	0.3157	0.9852	0.9570	1.0143
IP-10	-0.0005	0.9984	0.9995	0.6270	1.5934	0.0049	0.0531	1.0049	0.9999	1.0099
MCP-1	0.0856	0.4049	1.0894	0.8906	1.3326	-0.2965	0.0992	0.7435	0.5226	1.0575
MIP-1α	-0.1895	0.3722	0.8273	0.5457	1.2545	0.0639	0.7731	1.0660	0.6903	1.6461
MIP-1β	0.1054	0.0990	1.1112	0.9804	1.2595	-0.0463	0.4618	0.9548	0.8441	1.0800
PDGF-BB	-0.0012	0.2900	0.9988	0.9965	1.0011	**-0.0037**	**0.0316**	**0.9963**	**0.9930**	**0.9997**
RANTES	-0.0007	0.1152	0.9993	0.9984	1.0002	-0.0010	0.0572	0.9990	0.9981	1.0000
TNF-α	-0.1612	0.3417	0.8511	0.6105	1.1866	-0.3491	0.1528	0.7053	0.4371	1.1382
VEGF	0.0022	0.7425	1.0022	0.9890	1.0157	-0.0395	0.1177	0.9613	0.9149	1.0100
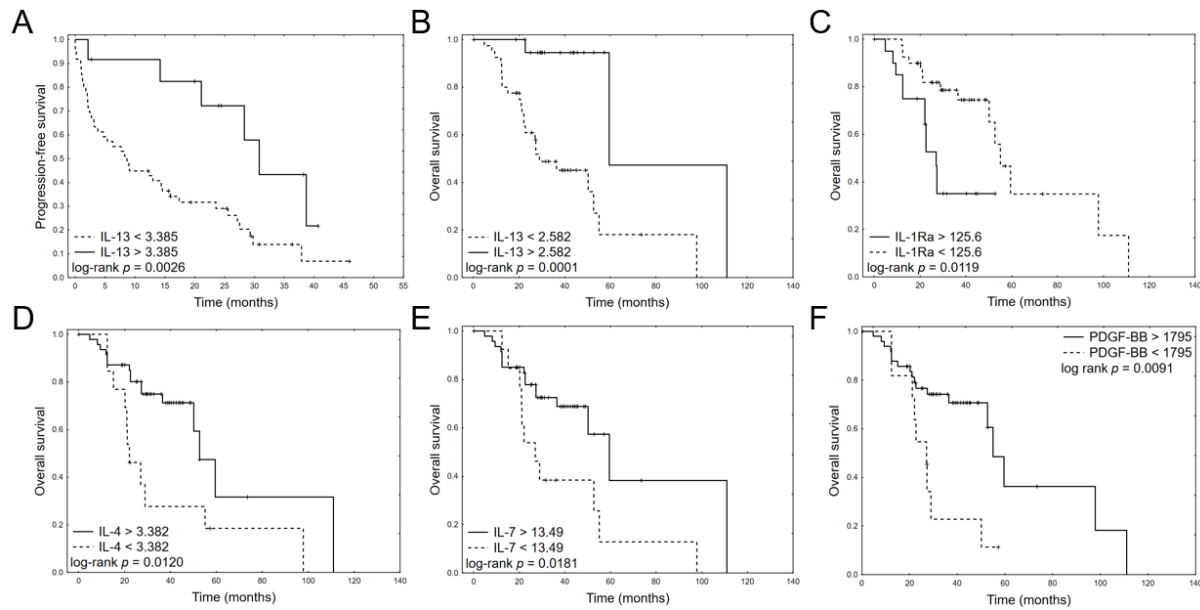
Figure 2. Kaplan–Meier plots for dichotomized significant cytokines in univariate analysis- PFS- IL-13 **(A)** and OS: IL-13 **(B)**, IL-1Ra **(C)**, IL-4 **(D)**, IL-7 **(E)**, PDGF-BB **(F)**.
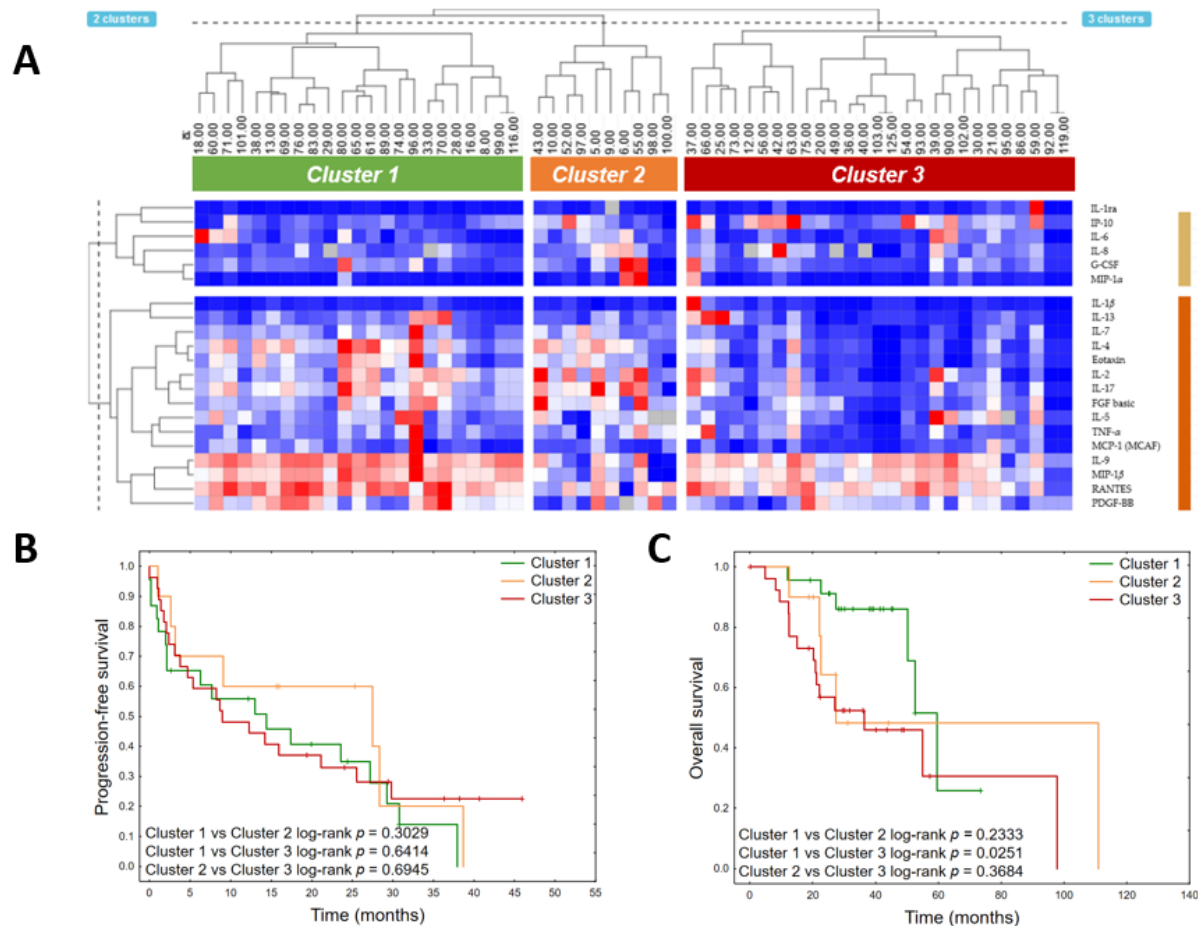
The cytokines found to be significant in univariate analysis were entered into a multivariate model building with a stepwise backward Akaike information criterion (AIC) elimination procedure, together with well-established prognostic factors (ISS III, ASCT, age >70, and presence of bone disease).   Briefly, an optimized model is identified by creating multiple models, initially including all selected variables. The variable with the highest p-value is eliminated at each step, and an AIC value is calculated for the new model; this process is repeated until no variables remain. The final multivariate models for PFS and OS with the lowest overall AICs are given in Table 4. The analysis confirmed that cytokine levels retained their importance in the simultaneous context of the clinical prognostic factors. The final model for PFS consisted of two variables: IL-13 cytokine level (HR 0.141, 95% CI: 0.024-0.829, p = 0.0302), and ASCT (HR 0.3722, 95% CI: 0.1826-0.7585, p = 0.0065). The final model for OS included ASCT (HR 0.142, 95% CI: 0.046-0.438, p = 0.0007), presence of bone disease at diagnosis (HR 3.826, 95% CI: 1.471-9.949, p = 0.0059) and two cytokine levels- IL-1Ra (HR 1.017, 95% CI: 1.0004-1.030, p = 0.0091) and IL-4 (HR 0.161, 95% CI: 0.037-0.698, p = 0.0147).

Table 4.   Final multivariate Cox regression analyses for PFS and OS of MM patients. Cytokine concentrations (pq/ml)   were divided by 10 to allow a more straightforward interpretation of coefficient values.

Variable	Coefficient	*p*	HR	95% CI	
				Lower	Upper
PFS					
IL-13	-1.958	0.0302	0.1411	0.0240	0.8291
ASCT	-0.494	0.0065	0.3722	0.1826	0.7585
OS					
IL-1Ra	0.017	0.0091	1.017	1.004	1.030
IL-4	-1.828	0.0147	0.161	0.037	0.698
ASCT	-0.975	0.0007	0.142	0.046	0.438
bone disease	0.671	0.0059	3.826	1.471	9.949

3.4. Cluster analysis

A two-way hierarchical cluster analysis was performed to classify cytokine patterns among multiple myeloma patients (Figure 3A). Six cytokines were excluded from the analysis due to high missing values (>15%): GM-CSF (n=41 values below limit of detection), IL-12(p70) (n=39), IL-10 (n=27), IL-15 (n=25), IFN-γ (n=18) and VEGF (n=10). One patient (ID 50) was excluded, as eleven cytokines in his sample were below the limit of detection.

Figure 3. Heatmap with hierarchical clustering performed on cytokine values **(A)** to identify multiple myeloma patient groups. Expression levels of individual cytokines are represented by shades of blue to red in the central heatmap, with the highest values in dark red and the lowest in dark blue. Kaplan–Meier plots of progression-free survival **(B)** and overall survival **(C)** each identified cluster of patients.

Two main clusters of cytokines were identified and three clusters of patients. The first cytokine clusters consisted of IL-1ra, IP-10, IL-6, IL-8, G-CSF and MIP-1α. The second consisted of IL-1β, IL-13, IL-7, IL-4, Eotaxin, IL-2, IL-17, FGF basic, IL-5, TNF-α, MCP-1 (MCAF), IL-9, MIP-1β, RANTES and PDGF-BB. The detailed level of each cytokine in particular clusters is provided in Table S1.

The patients in the first cluster were more frequently ISS III stage (p = 0.0287). Comparison of clinical variables between clusters is detailed in Table S2. Furthermore, patients in the third cluster had significantly shorter OS (HR 2.988 95% CI: 1.1454-7.7444, p = 0.0252) (Figure 3B, C).

4. Discussion

Previous studies have showed that different cytokines take part in the pathogenesis, progression, and prognosis of MM [12, 17].   The present study evaluated the prognostic significance of 27 cytokine serum levels in 61 previously-untreated MM patients receiving bortezomib-based regimens as first-line treatment. Furthermore, three clusters of patients were identified with different overall survival based on cluster analysis.

We observed that a higher serum pretreatment level of IL-13 is an independent predictor of longer PFS in MM patients treated with bortezomib-based chemotherapy. IL-13 is an anti-inflammatory Th2-type cytokine that suppresses the cytotoxic actions of macrophages, inhibits the production of pro-inflammatory cytokines, and plays a significant role in various inflammatory diseases [18]. Moreover, IL-13 plays a role in human osteoclast formation in a lymphocyte-dependent manner [19]. On the other hand, Di Lulo et al. [20] strongly suggest a role for IL-13 in MM progression through upregulation of adhesion molecules and IL-6 secretion by bone marrow mesenchymal stromal cells, which promotes MM cell growth. Previous studies have found that IL-13 upregulates VCAM-1 expression on endothelial cells   and increases adhesion molecule expression and IL-6 secretion in fibroblasts [21-23]. These observations suggest that bone marrow-derived mesenchymal stem cells (BM-MSCs) can be   targets for IL-13 in MM [20]. IL-13 increases adhesive molecule expression and IL-6 secretion by BM-MSCs; these cells demonstrate a similar response to IL-13 stimulation as other cells, including stromal fibroblasts [22]. It has been documented previously that IL-13 is involved in the stimulation of macrophages for antitumor activity. The IL13-activated macrophage subset is characterized by abundant amounts of macrophage C-type lectin receptors (CLRs) [24,25]. In the preclinical study IL13 inhibited T-cell lymphoma and ovarian adenocarcinoma development in mice through the conversion of tumor-supporting macro-phages to cytotoxic effectors [25]. The prognostic value of serum IL-13 level has been evaluated previously in other hematological malignancies. Özyörük et al [26] report higher serum IL-13 levels in children with lymphoma diagnosed with Hodgkin lymphoma or Burkitt's lymphoma; however, unlike the present study, they did not find this cytokine to have any prognostic significance.

Among the 27 tested cytokines, IL-1Ra, IL-4, IL-7, IL-13 and PDGF-BB were found to be predictors of OS in MM patients treated with bortezomib-based regimens in univariate models, and IL-1Ra and IL-4 maintained this significance in the multivariate model. However, among the MM patients treated with bortezomib, only IL-4 predicted longer OS in both the univariate and multivariate models: Patients with higher level of IL-4 had longer OS (Fig. 2). In normal conditions, IL-4 induces TH2 cell, B-cell, mast cell and eosinophil proliferation, as well as isotype switching for IgE production [27]. Kyrstsonis et al. found that while IL-4 levels were low (median 4 pg/ml) at diagnosis in 75% of MM patients and then rose in remission (median 25 pg/ml), IL-4 values remained stable during the course of the disease in chemotherapy-resistant patients [28]. In addition, Herrmann et al. observed reduced   plasma cell growth in MM patients treated with IL-4, probably by inhibition of endogenous IL-6 synthesis [29].

In our study, a high level of IL-1Ra correlated with shorter OS (Fig. 2). IL-1Ra is an anti-inflammatory acute-phase protein that competitively inhibits IL-1 activity and specifically inhibits paracrine IL-6 production [30,31]. Previous research has found higher levels of IL-1RA to be associated with bone involvement [12], and MGUS/SM/MM patients have demonstrated significant increases in serum IL-1Ra levels compared to healthy controls [32]. In addition, MM patients have been found to demonstrate elevated IL-1Ra levels in the bone marrow (BM) environment [33] and that IL-1Ra is produced by MM cells. Low post-transplantation IL-1Ra levels have also been found to correlate with engraftment syndrome in patients with plasma cell dyscrasias such as POEMS (polyradiculoneuropathy, organomegaly, endocrinopathy, M-spike, skin changes) [34]. IL-1Ra is a specific blocker of IL-1, which is a crucial factor in the induction of IL-17-producing T-cells in vivo [35].

A phase II trial of patients with smoldering or indolent MM showed improvement in PFS and OS duration after targeted treatment with IL-1Ra (Anakinra), with or without dexamethasone [36]. It was found that IL-1Ra bound to the myeloma proliferative cells and decreased the level of C-reactive protein (CRP), a surrogate for IL-6 production. Seven patients treated with IL-RA alone demonstrated a decrease in the plasma cell labeling index (PCLI) and three patients achieved a minor response (MR) to IL-1Ra alone.

When dexamethasone was added, an additional nine patients achieved a PR/MR. This study suggested that IL-1Ra, as a specific inhibitor of IL-1, induced paracrine IL-6 production and was effective in destroying the proliferative myeloma component.

IL-7 and platelet derived growth factor-BB (PDGF-BB) were also found to be significant predictors of OS in univariate analyses. IL-7 is a cytokine secreted by bone marrow stromal cells. It was previously observed that IL-7 prevents osteoblast formation by decreasing the activity of Runx2/Cbfa1 which is a transcription factor required for osteoblast differentiation [29]. Nierste et al. confirmed the presence of elevated levels of Dickkopf-1 (Dkk-1) and IL-7 in MM patients, and that these were responsible for the osteoblast differentiation from immortalized bone marrow mesenchymal stem cells (MSCs) [37]. In addition, they found that inhibition of Dickkopf-1 (Dkk-1) and IL-7 from MM plasma restored proper osteoblast differentiation in the MSC line. The IL-7 levels did not return to baseline levels in MM patients who are in remission [10].

In the present study, higher platelet-derived growth factor (PDGF)-BB serum level was associated with longer OS in bortezomib-treated patients in univariate analysis. PDGF is an angiogenic factor that can be formed by two A subunits (PDGF-AA), two B subunits (PDGF-BB), or one A and one B (PDGF-AB). PDGF influences c-myc gene expression through the c-myc promoter in a Src-dependent manner [38]. In an in vitro study, PDGF-BB was found to up-regulate Myc expression and reduce the melphalan sensitivity of tumor cell clones. Moreover, down-regulation of c-Myc protein induced the expression of PDGF-beta receptor molecules and decreased PDGF-BB release. Similarly, an in vivo study found melphalan-resistant MM patients to present overexpressed c-Myc protein and higher serum PDGF-BB receptor levels compared to minor responding patients.

The relationship between pretreatment cytokine serum levels and OS in newly-diagnosed MM patients has also been investigated in previous studies. Cheng et al. [39] explored macrophage inflammatory protein 1 alpha (MIP-1α), migration inhibitory factor (MIF), tumor necrosis factor-α (TNF-α), vascular endothelial growth factor-α (VEGF-α), monocyte chemoattractant protein-1 (MCP-1) and soluble interleukins IL-17A, IL-6, IL-21 and IL-10 before treatment. The authors were able to develop a prognostic nomogram using three variables, viz. lactate dehydrogenase (LDH), MIP-1α and creatinine levels, which accurately predicted the 1-year, 2-year and 3-year OS of MM patients.

Elsewhere, a retrospective analysis by Gu et al. [17] examined the serum levels of seven cytokines in patients with newly-diagnosed MM (IL-2, IL-4, IL-6, IL-10, and IL-17A, TNF-α, and IFN-γ). Univariate and multivariate analyses found that that serum IL-6 level >3 pg/mL, serum IL-17A level >4 pg/mL and treatment regimens were independent prognostic factors for PFS and OS. However, IL-4 serum level had no prognostic value, and IL-13 and IL-1RA were not included in the panel of evaluated cytokines in this analysis [17]. In another study, high serum IL-10 was found to predict poor prognosis [40]: the low-IL-10 group (⩽169.96 pg ml-1) was found to have an OR rate of 79.2% and the high IL-10 group (>169.96 pg ml-1) 53.3% (P<0.001). In addition, the patients in low-IL-10 group had significantly better PFS (3-year PFS rate: 69.3% vs 13.3%, P<0.001) and OS (3-year OS rate: 93.6% vs 51.9%, P<0.001) than the high-IL-10 group.

Wang et al. found that higher serum level of soluble interleukin-2 receptor (sIL-2R) predicts treatment resistance and poor outcome [41]. The level of sIL-2R in serum was measured using enzyme-linked immunosorbent assay (ELISA) in 81 patients with newly-diagnosed MM; however, in this study, only 26 of 81 (32.1%) patients included for the analysis were treated with bortezomib-based regimens, and 55 patients (67.9%) received older regimens, without targeted drugs. Thirty-six patients (44.4%) were classified into the higher sIL-2R level group (> 6.049 ng/ml), and 45 patients (55.6%) into the lower group. The lower sIL-2R group demonstrated a higher overall response rate (ORR) (60.0%), compared to the higher group (41.7%) (p = 0.156). In addition, the multivariate survival analysis found Eastern Cooperative Oncology Group performance status score,

treatment response, and sIL-2R level to be significant independent prognostic factors for PFS, but none of these factors was useful for predicting OS. Importantly, a high level of sIL-2R correlated significantly with inferior PFS in patients treated with bortezomib-based regimens (p = 0.004). However, sIL-2R was not included in the panel of cytokines investigated in the present study.

The present study also analyzed the influence of clinical variables on PFS and OS in Cox regression models. Of the tested variables, only the use of autologous stem cell transplantation (ASCT) during the treatment schedule was found to influence both PFS and OS. These observations are in agreement with previous reports [42,43]. High-dose therapy with melphalan followed by ASCT prolongs PFS, even in the era of novel agents [44]. A meta-analysis incorporating large phase 3 trials from January 2000 to April 2017 found HDT/ASCT to be associated with superior PFS than standard-dose therapy (SDT). However, the effect of HDT/ASCT on OS remains ambiguous and was not observed in this meta-analysis [44]. In addition, bone disease at diagnosis also significantly impacted PFS, and bone lesions have been found to have a negative prognostic influence in several previous reports [45,46]. A recent meta-analysis of PET parameters found the presence of extramedullary disease, more than three focal lesions, and high FDG uptake to be associated with shorter OS and PFS [47].

5. Conclusions

Only IL-13 pretreatment serum level was found to significantly impact PFS in newly-diagnosed MM patients treated with bortezomib-based regimens. In addition, serum levels of five cytokines, IL-1Ra, IL-4, IL-7, IL-13 and PDGF-BB influenced OS in univariate analyses. However, only IL-1Ra and IL-4 were found to have independent prognostic value in multivariate analyses. Three clusters of MM patients were identified, with different cytokine profiles and different OS. Our findings indicate that cytokine signature may have a potential influence on the outcome of MM patients treated with bortezomib. However, the clinical and biological importance of these findings require further investigation.

Abbreviations:

ASCT – autologous stem cell transplantation

BM - bone marrow

BM-MSC - bone marrow-derived mesenchymal stem cells

CRAB - calcium elevated, renal failure, anemia, and bone lesions

CXCL1 - C-X-C motif ligand 1IL- interleukin

GM-CSF – granulocyte, monocyte colony stimulating factor

IFN – interferon

IL-1-Ra - IL-1 receptor antagonist

IMiDs - immunomodulatory drugs

IMWG - International Myeloma Working Group

LCD - light chain disease

MIP - macrophage inflammatory protein

MM - Multiple myeloma

MSC - mesenchymal stem cells

OS – overall survival

PC - plasma cells

PCM - plasma cell myeloma

PDGF - platelet derived growth factor

PFS – progression-free survival

PI – proteasome inhibitor

POEMS - polyradiculoneuropathy, organomegaly, endocrinopathy, M-spike, skin changes

RANTES - Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted

TGF-β - transforming growth factor β

TNF- tumor necrosis factor

VCD - bortezomib, cyclophosphamide, dexamethasone

VEGF – vascular endothelial growth factor

VMP - bortezomib, melphalan and prednisone;

VTD -bortezomib, thalidomide, dexamethasone

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Supplementary tables:

Table S1. Cytokine profile of three identified clusters of multiple myeloma patients. Data are presented as mean values and SD, and as p-values from the global ANOVA test and *post hoc* comparisons (Tukey's test), if the p-value of the global test is significant.

Table S2. Comparison of clinical variables between identified three clusters of multiple myeloma patients.