RESEARCH ARTICLE

Significance of Some Proliferation Markers and Some Prognostic Factors in Patients with Multiple Myeloma and their Impact on the Patients' Survival

Iman A. Abdelgawad[1]*, Noha H. Radwan[1], Roxan E. Shafik[1], Hala A. Shokralla[2]

Abstract

    <u>Background</u>: Multiple myeloma (MM) is influenced by genetic and micro-environmental changes. Malignant plasma cells produce an abnormal monoclonal immunoglobulin, as well as cytokines, such as IL-10 and IL-6 which stimulate cells of the bone marrow microenvironment (BMM) and cause dysfunction and failure of many organs. B cell activating factor (BAFF), IL6, IL10 are known to influence the growth & survival of the malignant clone. <u>Aim</u>: The objectives of the present study were to investigate the circulating levels of BAFF , IL-10 and IL-6 , correlate them with well-known parameters of disease activity in patients with MM, and to detect their impact on the patients' survival. <u>Materials and Methods</u>: This study was conducted on 89 newly diagnosed MM patients and seventy apparently healthy volunteers as a normal control group. BAFF, IL6, IL10 were measured by ELISA for both groups. Survival analysis was performed for all patients. <u>Results</u>: Studied markers were higher in the MM patients compared to the normal control subjects. Patients' survival was improved by high serum BAFF levels. <u>Conclusions</u>: High levels of BAFF were found to improve patients' survival. BAFF and IL-6 can be considered probable diagnostic markers for MM.

Keywords: Multiple myeloma, BAFF, IL6, IL10.

Introduction

    Multiple genetic and micro-environmental changes are known to contribute to the pathogenesis of the malignant proliferation of a plasma cell clone causing multiple myeloma (MM) (Kyle et al., 2003). Nearly 230,000 people are living with MM worldwide (globocan , 2012), with 47% five year survival-rate (multiple-myeloma/statistics, 2015). MM can cause impaired humoral immunity (80%), skeletal disease (70%), anemia (40%), and renal impairment (20%) (Sirohi et al., 2004).

    Malignant plasma cells produce an abnormal monoclonal immunoglobulin, as well as cytokines, such as IL-10 and IL-6 which stimulate cells of the bone marrow microenvironment (BMM) and cause dysfunction and failure of many organs (Tricot ,2000).

    IL-10 is a powerful inducer for immunoglobulin secretion by the plasma cells. It is produced by activated T cells, monocytes, and mostly by normal and malignant B cells (Moore et al., 1993). It acts as a proliferation factor for B cells, and is involved in their differentiation to plasma cells. Furthermore, it seems to act as a growth factor for MM cells (Kovaks et al., 2010). IL-10 has been implicated in the pathogenesis of other malignant B-cell neoplasms as chronic lymphocytic leukemia (Gupta et al., 2012).

    IL-6 is produced by B cells, T cells (Horii et al., 1988), fibroblasts (Weissenbach et al.,1980) monocytes (Aarden et al., 1987), and some tumor cells (Okada et al., 1988). It promotes the population expansion and activation of T cells, the differentiation of B cells, and regulation of the acute-phase response (Hirano et al., 2014). It is involved in autoimmunity, inflammation, and lymphoid malignancies (DuVillard et al., 1995) and acts as an important growth factor for MM by inhibiting apoptosis induced by growth factors withdrawal, and by triggering the expression of cell-death receptor Fas (Tinhofer et al., 2000).

    BAFF is a tumor necrosis factor (TNF) family member, essentially expressed by monocytes, dendritic cells, and some T cells (Okada et al., 1988). It is critical for maintenance of normal B-cell development (Horii et al., 1988) and is considered a survival factor for immature, naive, and activated B cells (Okada et al., 1988). It is produced as both a membrane-bound and a soluble protein (Weissenbach et al.,1980). Myeloma cells express BAFF and its receptors (Okada et al., 1988) that are primarily produced by the BMM (Moreaux et al., 2004).

    Immunoglobulins (Igs) are continually produced throughout B-cell development and their terminal differentiation into plasma cells. Immunoglobulins are made up of mirror image identical light and heavy chains. The amount of κ light chain produced is almost twice that

[1]*Clinical Pathology department,* [2]*Medical Oncology department, NCI, Cairo University Cairo, Egypt *For correspondence: imanaga70@yahoo.com*

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of λ (Bradwell et al., 2001).

Aim of the work: Is to compare the serum levels of BAFF, IL-10 and IL-6 between the MM patients and the normal control group. Also to study their impact on the survival of the MM patients. Comparing the tested markers with some prognostic factors of MM is also one of the aims of this study.

Materials and Methods

Patients

This study was conducted on consecutive 89 newly diagnosed MM patients who presented to the outpatient clinic at the medical oncology department, National Cancer Institute, Cairo University. All eligible new cases were included in the study. The study lasted 3 years from January 2012 till December 2014. All patients were proven to have MM according to the standard criteria of the International Myeloma Working Group (IMWG) (Bird et al., 2011).

Seventy apparently healthy volunteers were included in the study as a normal control group.

The study protocol was approved by the Scientific Research Committee and Institutional Review Board at the National Cancer Institute, Cairo University, Egypt.

Written informed consent was obtained before enrollment into the study according to ethics committee.

Inclusion criteria:

Patients proven to have MM.
Newly diagnosed patients prior to any therapeutic intervention.
Either sex was eligible.
Age: 18-70 years.
Performance status (ECOG Scale) ≤ 2 (Oken et al., 1982).
Life expectancy of at least 6 months.

Exclusion criteria

Patients with serious concomitant systemic disorders, current or previous other malignancies at time of diagnosis.
Patients with uncontrolled infectious diseases.
Treatment was initiated in all patients with active myeloma fulfilling the CRAB criteria, (hypercalcaemia >11.0 mg/dl), creatinine >2.0 mg/ml, anaemia (Hb<10 g/dl), active bone lesions, and in those symptomatic due to the underlying disease in form of VAD (vincristine, adriamycin& dexamethasone) or VD (bortezomib & dexamethasone) (Moreau et al., 2013).

Methods

Serum samples were obtained from all control subjects and from the patients prior to any therapeutic intervention, and were subjected to the following:
Kidney function tests, calcium, alkaline phosphatase, total proteins, albumin, and β2m using Beckman CX9 auto-analyzer, Switzerland.
Measurement of BAFF, IL-10 and IL-6 were done according to the manufacturer's instructions using Human BAFF Instant ELISA kit 2007INST eBioscience, Human IL-10 Platinum ELISA kitBMS215/2/BMS215/2TEN

eBioscience, and Human IL-6 Platinum ELISA BMS213/2/BMS213/2TEN eBioscience, Vienna, Austria respectively.

Complete blood counts were performed on blood specimen preserved on EDTA using coulter counter, Switzerland.

All patients were subjected to bone marrow (BM) aspiration, counting and interpretation, while BM biopsy was done for patients giving dry tap.

Serum protein electrophoresis (SPE) was performed to detect the presence of M band using Sebia-hydrasys semi-automated electrophoresis system.

Immunofixation electrophoresis

IFE testing was performed for identifying immunoglobulin light and heavy chains using equipment purchased from Sebia (Sebia electrophoresis, HYDRASYS) and reagents (Hydragel IF; acid violet). This assay was performed according to the manufacturer's guidelines. Electropherograms were scanned and followed by final assessment and clinical interpretation.

Serum calcium concentrations were corrected when serum albumin was <4 g/dL using the following equation:
Corrected calcium (mg/dl) = Serum calcium (mg/dL) + 0.8 (4 - serum albumin g/dL) (Carl et al., 1999).

Statistical analysis

Data analysis was done by using IBM SPSS advanced statistics version 20 (SPSS Inc., Chicago, IL). The descriptive measures were presented in frequency and percentages. For quantitative data, comparison between two groups was done using Mann-Whitney (non-parametric t-test), and between 3 groups, Kruskall Wallis (non-parametric ANOVA) was used. The Receiver Operating Characteristic (ROC) curve was used for prediction of cut off values. Sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) were calculated for the different markers used. Survival analyses were done using the Kaplan–Meier method. Comparisons between two survival curves were done using log-rank test. Overall survival (OS) was defined as the time in months between the date of diagnosis and death or loss to follow up. Disease -free survival (DFS) was defined as the time in months between the date of treatment and documented recurrence, progression or death. Cut off values used for the prediction of patients' survival were chosen as the value of the median level of the measured variable among the malignant cases. P-value of ≤ 0.05 was considered significant.

Results

Patients' characteristics are mentioned in Table (1).

On Comparing the serum levels of IL-10, BAFF, IL-6, and β2M between the MM and the normal control groups, all the tested markers were statistically significantly higher in the MM compared to the normal control group (p <0.001) each. Differences in clinical parameters according to albumin, β2M and IL-6 levels in the MM patients are shown in Table (2), while the diagnostic performance of the different studied markers is shown in Table (3).

On comparing some prognostic factors of multiple

Proliferation Markers in Multiple Myeloma

myeloma as β2m, albumin, creatinine, hemoglobin, BAFF, IL-10 and IL-6 according to the type of immunoglobulin, type of light chain, and the stage of the disease; creatinine, BAFF, and IL-6 significantly showed the highest

concentrations with the presence of lambda compared to kappa light chains (p=0.009, 0.004, and 0.003) respectively. Higher levels of β2m and IL-6 and lower levels of albumin and hemoglobin were significantly associated with advanced stage (p<0.001) each. Higher levels of creatinine and lower levels of hemoglobin were significantly associated with IgA MM compared to IgG MM (p=0.05 and 0.013) respectively.

As regards survival, the different diagnostic and prognostic parameters were studied in relation to both (OS) and (DFS).

Overall survival (OS)

The follow up period ranged between 1 and 36 months with a median OS not reached. At the end of the study (3 years), OS was 74%, and at 5 years, 72.7% are expected to be still alive.

Eighty five % of patients with high BAFF levels (>1.38 ng/ml) were still alive after a total period of 24 months compared to only 64 % of patients with low BAFF levels (Figure 1). So, BAFF level could be considered a strong predictor for OS in MM (p<0.023).
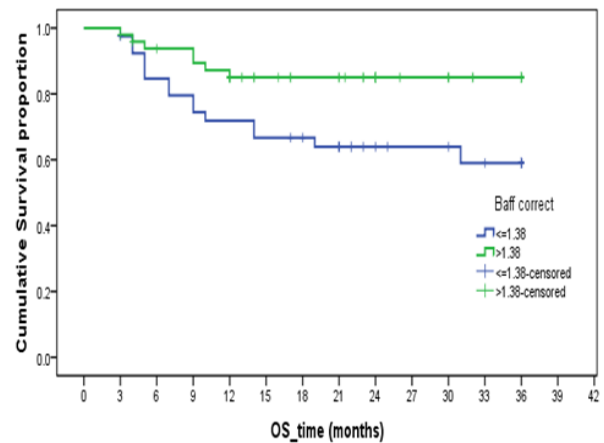
Table 1. Patients' Characteristics

Variable	N (%)
Age (median and interquartile range)	
54 (45-59)	
Sex	
Male	56 (63)
Female	33 (37)
Albumin	
< 3.5 g/dl	38 (43)
≥ 3.5 g/dl	51 (57)
Serum creatinine	
<1.2 mg/dl	58(65)
≥1.2 mg/dl	31 (35)
β2m	
<2.7 mg/L	24 (27)
≥ 2.7 mg/L	65 (73)
Corrected calcium	
< 10.5 mg/dl	81 (91)
≥ 10.5 mg/dl	8 (9)
Type of immunoglobulin	
IgG	73 (82)
IgA	16 (18)
Type of light chain	
Kappa	58 (65)
Lambda	31 (35)
Type of Ig with corresponding light chain	
IgG - K	51 (57)
IgG- λ	22 (25)
IgA-K	7 (8)
IgA- λ	9 (10)
MM Staging (ISS)	
I	22 (25)
II	20 (22)
II	47 (53)
Plasma cell %	
<30	22 (25)
≥30	67 (75)

*IgG: Immunoglobulin G; IgA: Immunoglobulin A; K: Kappa; λ: Lambda; MM: Multiple myeloma; ISS: International staging system

Figure 1. Relation between BAFF Concentration and Overall Survival

Table 2. Differences in Clinical Parameters According to Albumin, β2M and IL-6 Levels in the MM Patients

	Albumin < 3.5 g/dl (n=38)	Albumin ≥ 3.5 g/dl (n=51)	P value	β2M <2 mg/l (n=14)	β 2M ≥ 2 mg/l (n=75)	P value	IL-6 <7.4 pg/ml (n=16)	IL-6 ≥7.4 pg/ml (n=73)	P value
Albumin				3.9	3.5	0.271	4	3.4	0.015*
(3.5 – 5.2g/dl)				(3.2 - 4.5)	(2.8 - 4.2)		(3.8 - 4.4)	(2.7 - 4)	
Hb conc	9.2	10.9	0.004*	11.6	9.5	0.01*	12	9.4	0.002*
	(8 - 10.6)	(8.9 - 12.6)		(10.6 - 12.6)	(8.4 - 11.2)		(10.7 - 13)	(8.3 - 11)	
β2 M	6.9	5.2	0.09				2.2	7	<0.001*
(1.21-2.70 mg/l)	(3.9 - 10.4)	(2.2 - 7.8)					(1.7 - 2.6)	(4 - 9.7)	
Creatinine	0.97	0.9	0.208	0.89	0.93	0.163	0.75	1	0.011*
(0.2 – 1.2 mg/dl)	(0.8 – 1.9)	(0.7 – 1.3)		(0.7 - 1)	(0.75 - 1.6)		(0.65 - 0.99)	(0.8 - 1.6)	
BM plasma cells	17	13	0.881	7	17	0.033*	15	15	0.525
	(7 - 30)	(4.25 - 34)		(1 - 13)	(6 - 31)		(2.5 - 28)	(6 - 32)	

*Significant; Median and interquartile range in parenthesis; Hb: Hemoglobin; β2 M: Beta 2 microglobulin; BM: Bone marrow; IL-6: Interleukin 6; Hb conc in men: 14.0-17.5 g/dL; Hb conc in women: 12.3-15.3 g/dL

Table 3. Diagnostic Performance of the Different Markers for the Differentiation Between Multiple Myeloma and Normal Control Groups

	BAFF Cut off 1 ng/ml	IL-10 cut off 4.8 pg/ml	IL-6 cut off 4.2 pg/ml	β2M cut off 2 mg/l
Sensitivity %	100	91	99	87
95% CI	(95 - 100)	(83 - 96)	(98 - 99)	(78 - 93)
Specificity %	88	64	96	74
95% CI	(75 - 95)	(49 - 77)	(85 - 99)	(59 - 84)
PPV%	94	82	98	84
95% CI	(86 - 97)	(73 - 89)	(91 - 99)	(75 - 91)
NPV%	100	80	98	78
95% CI	(90 - 100)	(64 - 90)	(88 - 99)	(64 - 88)
DA%	95	82	97	82

β2 M: Beta 2 microglobulin; BAFF: B-cell-activating factor; IL 10: Interleukin 10; IL-6: Interleukin 6; CI: Confidence interval; PPV: Positive predictive value; NPV: Negative predictive value; DA: Diagnostic accuracy
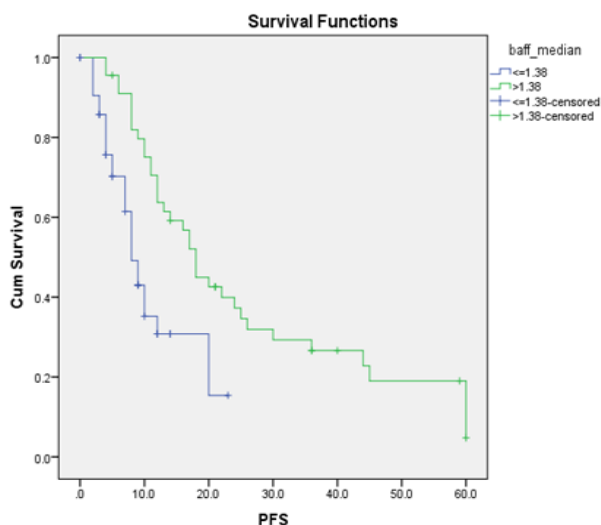

Figure 2. Relation between BAFF and DFS

Disease free survival (DFS)

The median DFS was 12 months; with 76% 3-years DFS and 45.7% at 5 years.

BAFF was also found to be strongly related to DFS (p<0.001). Median DFS of patients with higher levels (>1.38 ng/ml) was better than those with low levels (≤1.38 ng/ml) (18 vs. 8 months) as shown in figure (2). Lower levels of serum creatinine , plasma cell infiltration of BM and normal hemoglobin levels were associated with better DFS (p=0.01, 0.05 and 0.01) respectively.

β2m was found to be an independent prognostic factor for DFS (p=0.006). Median DFS of low β2M ≤2 mg/l was 36 months compared to only 11 months for patients with higher levels (p=0.02).

No other statistically significant relations were detected between survival and other prognostic factors of MM as; levels of ALP, albumin, calcium, IL 6, IL10, type of Ig and light chain, and stage of the disease.

Discussion

BAFF is suggested to stimulate the myeloma cells through an autocrine loop (Moreaux et al., 2004).

Depending on the B cell maturation stage, BAFF was reported to induce the anti-apoptotic proteins Bcl-2 and to reduce the pro-apoptotic protein Bak (Batten et al., 2000).

From all the studied proliferation markers, higher levels of serum BAFF (>1.38 ng/ml) were found to be significantly associated with longer OS and DFS among myeloma patients (p=0.023), which is contradictory to the results of other investigators, who suggested BAFF to be a possible prognostic factor and a strong predictor for OS in MM as it was associated with reduced survival (Lemancewicz et al., 2013, Hengeveld et al., 2015).These contradictory results may be due to the different ethnic population studied , or different kind of therapy received.

As BAFF regulates and enhances innate and adaptive immunity (Caers et al., 2008),it may lead to improvement of MM patients' survival which might support our results. BAFF has also been identified as one of the main survival factors for healthy plasma cells as well as MM cells (Caers et al., 2008). So, more studies with larger sample sizes are recommended to monitor BAFF serum levels before and after treatment, to investigate the predominant role of BAFF on both healthy and malignant plasma cells and to study the different signaling pathways to reach a more accurate conclusion about the impact of BAFF on the survival of MM patients.

Higher serum levels of BAFF together with IL-6, IL-10 and β2m were found to be significantly higher in MM patients compared to the normal control group (P<0.001), which falls in line with previous reports (Wang et al., 2011,Fragioudaki et al., 2012,Alexandrakis et al., 2013). Also, IL-6 had the best diagnostic performance of the tested markers, followed by BAFF.

Higher BAFF levels together with IL-6 and serum creatinine were also detected in patients with lambda light chain (P=0.033) compared to patients with kappa light chain, although, normally, the amount of ϰ light chain produced is almost twice that of λ (Bradwell et al., 2001). The structural differences between both types may relate to their different biological activities and relations with other prognostic factors, as kappa light chains are described as monomeric, which can exist as a non-covalently linked dimer, while λ light chains are usually dimeric with covalent bonds between them (Solomon et al., 1976).

Regarding the type of immunoglobulin, IgA MM was significantly associated with higher levels of creatinine, and lower levels of hemoglobin compared to IgG MM.

Evidence indicates that IgA myeloma is different from

IgG myeloma in terms of disease characteristics (Sirohi et al., 2001). In an international study of staging in myeloma, IgA patients had a median overall survival of 40 months compared with 49 months for IgG patients; the reason for this difference was not explored (Greipp et al., 2005).

β2m was found to be an independent prognostic factor for DFS as higher levels significantly reduced median DFS (p=0.02). It was significantly higher with advanced stage (P<0.001). It seems that β2m has a negative impact on the patients' outcome as it reflects the tumor burden and increases in case of renal insufficiency (Pika et al., 2008). Some BJ MM cases are associated with a-more rapid onset of tubular and glomerular damage (Solomon, 1986) resulting in a reduced glomerular filtration rate and diminished clearance of β2m.

Anemic patients, higher plasma cell counts and higher serum creatinine levels showed their negative impacts on DFS as previously shown in other publications (Bird et al., 2011).

Albumin and β2m are routinely used to classify MM. Serum β2M reflects not only tumor mass and renal function but also some immune functions (Durie et l., 2003). IL-6 was suggested to be a significant prognostic marker in MM based on its strong correlation with other prognostic factors (Lauta et al., 2003), which supports our finding that IL-6 was significantly higher with advanced stage.

We divided the MM group according to the serum levels of albumin, β2m, IL-6, IL-10 and BAFF at the chosen cut off levels into 2 groups and compared both groups according to some prognostic factors of MM (hemoglobin, ALP, BM plasma cell count, type of immunoglobulin, and type of light chain). Higher albumin levels (≥3.5 g/dl) were significantly associated with higher hemoglobin concentrations. Lower β2m concentrations (< 2 mg/l) were significantly associated with higher hemoglobin concentrations and lower BM plasma cell counts, while lower levels of IL-6 (< 7.4 pg/ml) were significantly associated with higher albumin and hemoglobin and lower β2m, and creatinine levels. So, albumin, β2m and IL-6 are suggested to be included in a panel to be used for classification and prognosis stratification among MM patients, which needs further studies on a wider scale to validate such results. These results are consistent with other investigators (Kim et al., 2010).

In conclusion, High serum levels of BAFF were found to improve patients' survival. Further studies are still needed to validate these results. The presence of high levels of β2m was found to impact the patients' survival which is matching to our expectations as β2m reflects the tumor burden.

Albumin, β2m and IL-6 are suggested to be included in a panel to be used for classification of MM due to their significant relations with many prognostic factors.

More studies including larger sample size and longer durations of follow up are recommended to validate such results.

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100.0

6.3

75.0

56.3

50.0

25.0

31.3

0

Newly diagnosed without treatment