Research Paper Physical Education

The Impact of Bone Marrow *LMO2* (Lim Domain Only2) Protein Expressions on the Clinical Course of Plasma Cell Myeloma

* Engin Kelkitli ** Hilmi Atay *** Levent Yıldız **** Mehmet Turgut

* Department of Hematology, Erzurum Region Training and Research Hospital, Erzurum, Turkey

** Department of Hematology, Van Training and Research Hospital, Van, Turkey

*** Department of Pathology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey

**** Department of Hematology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey

ABSTRACT

The aim of this study was to evaluate whether the LMO2 protein expression can predict the clinical outcome in plasma cell myeloma (PCM) patients. LMO2 has been defined as an oncogene associated with carcinogenesis and LMO2 had better prognosis in several malignant tumors. Forty-one patients with PCM, 21 were male and 20 were female. The mean age was 62±11(min-max: 39-81). Thirty patients had LMO2 protein expression in bone marrow samples whereas in eleven patients LMO2 protein expression was not detected. The risk of death was increased in the patients with LMO2 protein expression compared with patients who had no LMO2 protein expression. While overall survival was %100 in the patients with no LMO2 protein expression, it was 76,7% in the patients with LMO2 protein expression (p=0.04). The risk of death was increased in the patients with LMO2 protein expression compared to the PCM patients who had no LMO2 protein expression.

Keywords : LIM domain only 2 (LMO2) protein, bone marrow, plasma cell myeloma, survival

Introduction

LIM-domain proteins are a large family of proteins that are emerging as key molecules in a wide variety of human cancers. *LMO2* (LIM domain only 2), which belongs to a family of four genes encoding LIM only proteins and are transcription regulators that control cell fate in normal hematopoiesis and endothelial cell remodeling, are located on 11p13. In particular, all members of the human LIM-domain-only (*LMO*) proteins, *LMO1–4*, which are required for many developmental processes, are implicated in the onset or the progression of several cancers, including T-cell leukemia, breast cancer, and neuroblastoma (1,2).

A malignant disease caused by the proliferation of single plasma cell clone derived from B cells is called plasma cell myeloma (PCM). An understanding of the biology of myeloma paved the way for new treatments that target myeloma cells that play a critical role in the pathogenesis of the disease and microenvironments of the bone marrow (3).

LMO2 has been defined as an oncogene associated with carcinogenesis, and *LMO2* had better prognosis in several malignant tumors such as pancreatic and prostate cancer (4). The *LMO2* protein was shown to be over-expressed in diffuse large B-cell lymphoma of the germinal center type, but this correlated with improved survival (2,5). In the review of the literature, no studies were found that investigated the prognostic value of *LMO2* expression in patients with PCM. Therefore, this study examined whether *LMO2* protein expression can predict the clinical outcome in PCM patients.

Material and Methods

This study included PCM patients with newly diagnosed, relapsed, or active disease between January 2005 and December 2011 according to the International Working Group, and treated bortezomib containing regimes. The latest update was conducted in January 31, 2012. Prior to this study, 19 Mayıs University School of Medicine Ethics Committee approval was obtained with Number 885 on January 27, 2012. Forty-one patients with PCM and admitted to our hospital were enrolled in the study. Immunohistochemistry on bone marrow biopsy material for *LMO2* protein was performed in our hospital pathology laboratory, and staining in greater than 30% of plasma cells was assigned positive score (5,6) (Figures 1 and 2). As the young myeloid cells has a strong positive staining with LMO2, it was confirmed by staining the plasma cells with the plasma cell markers (CD38,CD138) at the same time.

Briefly, after a 4-6 hour decalcification process, trephine biopsy materials of the cases were detected in Gooding-steamed solution. Paraffin tissue blocks were prepared through routine tissue tracking each night (LeicaAsp). Four to six µm sections obtained from the paraffin blocks were stained with *LMO2* primary antibody and an immunohistochemical study was performed (Santra CruzBiotechnology, California ,USA. Clone 1A9-1,Lot≠ L1007) Anti *LMO2* antibody was used at a dilution of 1:150. Detection was carried out using the Vantana Benchmarc XT automatic staining system. For LMO2, thymus tissue was used for the positive control and non-immune serum was used as the negative control. The prepared sections were examined by a hematopathologist. Over 30% of plasma cells stained were considered positive. OS was calculated as time from randomization until death from any cause.

Statistical analyses

The Kaplan-Meier method was used to calculate survival curves and statistical comparisons were performed by the log-rank test. The chi-square test was used to analyze the quantitative data. ***P*** values <0.05 were considered significant.

Results

Of the 41 patients, 21 were male and 20 were female. The mean age was 62±11 years (min-max: 39-81). The demographic characteristics of patients are shown in Table 1.

Thirty patients had LMO2 protein expression in bone marrow samples, whereas in eleven patients LMO2 protein expression was not detected. LMO2 negativity was more frequent in patients with a type of lambda M protein (P=0.04)

The follow-up period ranged from 3 to 68 months (median: 19 months), and seven patients (20.5%) died. The relationships between LMO2 protein expression and patient clinical outcomes were examined (Figure 3). The risk of death increased in patients with LMO2 protein expression compared to patients who had no LMO2 protein expression. While overall survival was 100% in the patients with no LMO2 protein expression, it was 76.7% in patients with LMO2 protein expression (according to the Kaplan Meier test, long rank p=0.04)

Discussion

This study indicates the importance of the prognostic value of LMO2 expression at the time of diagnosis in patients with PCM. The results of this study demonstrate that the duration of overall survival was shorter in patients with PCM who had positive LMO2 protein expression at the time of diagnosis.

LMO2 was shown to be necessary for the development of normal hematopoiesis and plays a role in the development of all bone marrow derived hematopoietic lineage (7). In recent years, the investigations reporting the prognostic importance of LMO2 expression in malignancies has attracted attention (4,5,8,9,10). While marked advances have been made in establishing the significance and function of *LMO2* in T-ALL and B-cell lymphomas, B-cell acute lymphoblastic leukemia, and chronic myeloid leukemia (CML) and its role in plasma cell myeloma (PCM) has not been investigated.

LMO2 expression is also found in B-cell lymphomas derived from GC lymphocytes, including follicular, Burkitt's, and diffuse large B-cell (DLBCL) lymphomas, as well as in lymphocyte-predominant Hodgkin's lymphoma (5). In addition, *LMO2* expression is an independent prognostic factor of survival in patients with DLBCL treated with anthracycline-based chemotherapy with or without rituximab (11). Royer-Pokora B et al. (12) reported that high LMO2 protein expression is related to acute T-cell leukemia having the t(11:14)(p13:q11) and t(7:11)(q35:p13) translocations. Alizadeh et al. showed that LMO2 was expressed in the germinal center B like DLBCL, a DLBCL subtype with a better prognosis than DLBCL (13). A study performed by Cobanoğlu et al. (14) showed that the LMO2 protein is expressed in a significant proportion of B-ALL and AML, and the staining of LMO2 protein does not predict survival in acute leukemia. A study performed by Sönmez et al., LMO2 protein expression was studied with the immunochemical method with the materials at the time of diagnosis of bone marrow biopsy, hematologic remission in those LMO2 overexpressing CML patients treated with imatinib and reported that average life expectancy is better (8).

The current study demonstrated that the expected duration of survival for patients with LMO2 negative PCM was higher than the patients who were positive. In other words, the LMO2 expression in patients with PCM may be associated with survival. However, large scaled investigations are needed.

Agostinelli at al. (6) reported that B-ALLs, T-ALLs and acute myelogenous leukemia show intense and diffuse nuclear LMO2 positivity. On the other hand, LMO2 expression is low or absent in peripheral T-cell lymphomas, and other B-cell neoplasms, including chronic lymphocytic leukemia, hairy cell leukemia, and multiple myeloma. In another study by Natkunam at al. (5) reported that immunohistologic analysis of LMO2 protein expression in hematolymphoid neoplasia is a rare staining that was observed in the biopsies of myeloma (2%). The current studies' patients' positive staining was observed in approximately 73%. The results of this study demonstrate that LMO2 expression is high in PCM, contrary to the suggestions of by Agostinelli et al. and Natkunam et al. The high rate of LMO2 positive staining in the current study poses the question as to whether it could be related to the treatment-resistant disease. However, extensive studies in this subject are needed.

In conclusion, this study is the first in the literature showing the relationship between LMO2 protein expression and overall survival in PCM patients. We report that no LMO2 protein expression is correlated with improved overall survival in PCM patients. This method is easy, potent, and affordable. We suggest that the expression of LMO2 should be tested in a larger series of PCM patients.

Table 1: Demographic characteristics of patients

	n	LMO2 Positive n (%)	LMO2 Negative n (%)
Age	62 (39-81)		
Sex			
Male	20	17(85)	3(15)
Female	21	13(61,9)	8(38,1)
M protein Type			
IgG Kappa	12	7(58,3)	5(41,7)
IgA Kappa	8	4(50)	4(50)
IgG Lambda	12	12(100)	0(0)
Kappa	5	3(60)	2(40)
Lambda	4	4(100)	0(0)
Stage			
ISS stage I	3	1(33,3)	2(66,7)
ISS stage II	15	11(73,3)	4(26,7)
ISS stage III	23	18(78,3)	5(21,7)
Last state			
Exitus	7	7(100)	0(0)
Living	34	23(67,6)	11(32,4)
LMO2 Total		30(73,2)	11(26,8)

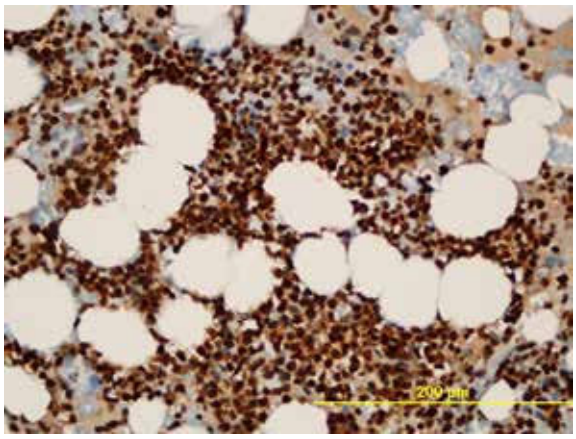

Figure 1: LMO2 positive stained bone marrow (LMO2X40 magnification).

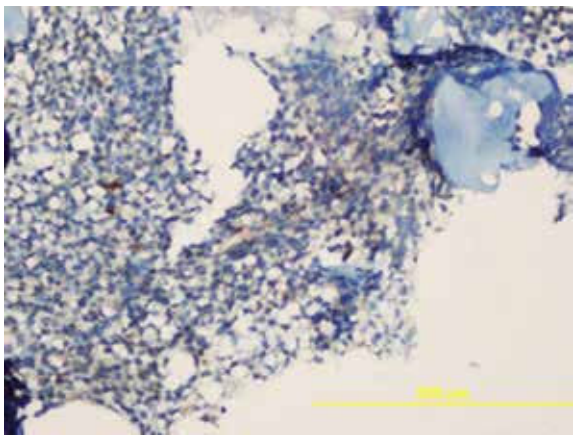

Figure 2: LMO2 negative stained bone marrow (LMO2X40 magnification).

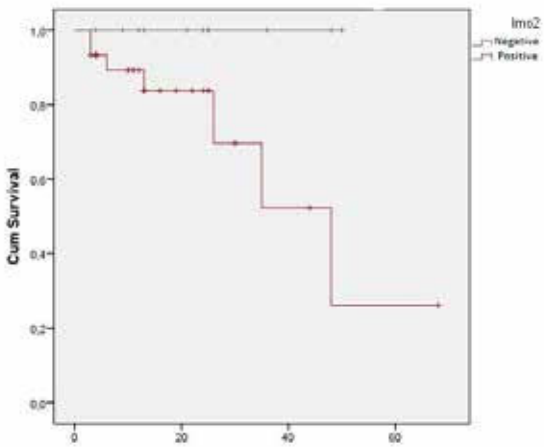

Figure 3: *LMO2* **expression predicts overall survival in patients with PCM.**

REFERENCES

1) Jacqueline M. Matthews, Krystal Lester, Soumya Joseph & David J. Curtis. LIM-domain-only proteins in cancer.Nature Reviews Cancer 2013;13: 111-122 | 2) Cerhan JR, Natkunam Y, Morton LM, et al. LIM domain only 2 protein expression, LMO2 germline genetic variation, and overall survival in diffuse large B-cell lymphoma in the pre-rituximab era. Leukemia Lymphoma. 2012; 53:1105-12. | 3) Tricot G, Fassas A. Multiple Myeloma and other plasma cell disorders. In Hoffman R,Benz JR EJ, Shattil SJ,Furie B, Cohen HJ Silberstein LE, Mcglave P editors. Hematology Basic Principles and Practice. Elsevier Churchill Livingstone, Philadelphia, USA. 2008;1501- 1535. | 4) Kohei Nakata, Kenoki Ohuchida, Eishi Nagai, et al. LMO2 Is a Novel Predictive Marker for a Better Prognosis in Pancreatic Cancer. Neoplasia. 2009 ; 11: 712–719. | 5) Natkunam Y, Zhao S, Mason DY, Chen J, Taidi B, Jones M, Hammer AS, Hamilton Dutoit S, Lossos IS, Levy R. The oncoprotein LMO2 is expressed in normal germinal-center B cells and in human B-cell lymphomas. Blood. 2007; 109:1636-42. | 6) Agostinelli C, Paterson JC, Gupta R, Righi S, Sandri F, Piccaluga PP, Bacci F, Sabattini E, Pileri SA, Marafioti T. Detection of LIM domain only 2 (LMO2) in normal human tissues and haematopoietic and non-haematopoietic tumours using a newly developed rabbit monoclonal antibody. Histopathology. 2012; 61:33-46. | 7) Yamada Y, Pannell R, Forster A, Rabbitts TH. The LIM-domain protein Lmo2 is a key regulator of tumour angiogenesis: a new anti-angiogenesis drug target. Oncogene. 2002; 21:1309-15. | 8) Sonmez M, Akagun T, Cobanoglu U, Topbas M, Erkut N, Yilmaz M, Ovali E, Omay SB. Effect of LMO2 protein expression on survival in chronic myeloid leukemia patients treated with imatinib mesylate. Hematology. 2009;14:220-3. | 9) Malumbres R, Fresquet V, Roman-Gomez J et al. LMO2 expression reflects the different stages of blast maturation and genetic features in B-cell acute lymphoblastic leukemia and predicts clinical outcome. Haematologica 2011; 96; 980–986. | 10) Nakata K, Ohuchida K, Nagai E, Hayashi A, Miyasaka Y, et al. LMO2 is a novel predictive marker for a better prognosis in pancreatic cancer. Neoplasia. 2009 ; 11: 712-9. | 11) Natkunam Y, Farinha P, Hsi ED, et al. LMO2 protein expression predicts survival in patients with diffuse large B-cell lymphoma treated with anthracycline-based chemotherapy with and without rituximab. J Clin Oncol. 2008; 26: 447-54. | 12) Royer-Pokora B, Loos U, Ludwig WD. TTG-2, a new gene encoding a cysteine-rich protein with the LIM motif, is overexpressed in acute T-cell leukaemia with the (11;14)(p13;q11).Oncogene 1991; 6: 1887–1893. | 13) Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000; 403:503-11. | 14) Cobanoglu U, Sonmez M, Ozbas HM, Erkut N, Can G. The expression of LMO2 protein in acute B-cell and myeloid leukemia. Hematology. 2010;15:132-4