Available online at www.medicinescience.org

ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2022;11(3):953-9

Comparison of the effects of C-KIT, MMP-2, Ki-67, Bcl-2 and metallothionein expression on prognostic factors in skin and non-skin malignant melanomas

Berna Eriten[1], Fahrettin Goze[2],

[1]Malatya Education and Research Hospital, Department of Pathology, Malatya, Turkey
[2]Sivas Cumhuriyet University, Faculty of Medicine Department of Surgical Medicine, Sivas, Turkey

Received 05 January2022; Accepted 26 April 2022
Available online 20.06.2022 with doi: 10.5455/medscience. 2021.05.174

Abstract

We aimed at the relationship between selected prognostic factors of multiple myeloma (MM), 1) C-KIT (CD117), which is related to MM pathogenesis. In this study, 30 skin and 15 non-skin MMM cases diagnosed between January 01, 2000 and June 01, 2011 in SCU-TF Medical Pathology Department were examined. C-Kit, MMP-2, Ki-67, Bcl-2 and MT markers were applied to these cases. When comparing the skin and non-skin groups, the difference between Ki-67 and MMP-2 expressions was found to be statistically significant, but no significant difference was found in staining with c-Kit, MT and bcl-2. It was thought that high MMP-2, C-KIT, bcl-2 expressions and high Ki-67 index as well as loss of MT expression might be associated with poor prognosis. Despite the high level of KIT immunostaining in melanomas, this parameter does not appear to be a good predictor of the presence of molecular mutations. Mutations that activate KIT should be considered a rare event in this tumor.

Keywords: bcl-2, c-KIT, kellgren-lawrence staging system (KLSS), Ki-67, malignant melanoma, metallothionein, MMP-2, prognostic factors

Introduction

Multiple Myeloma (MM) is one of the most important malignant tumors of the skin, with a high mortality rate and high invasion capacity. It can also arise from mucosal surfaces. These surfaces include oral and anogenital mucosa, sinonasal mucosa, esophagus, intestines, meninges, all layers of the eye and bladder [1-3].

Major prognostic factors of malignant melanoma are Breslow thickness, tumor size (largest tumor size and others), ulcer, histological type, Clark level in the skin, tumor infiltrating lymphocytes, lymphovascular invasion, perineural invasion, and regression. The first three of these (Breslow thickness, largest tumor size and ulcer) together with lymph node and distant organ metastases determine the pathological stage according to the TNM classification.

***Corresponding Author:** Berna Eriten, Malatya Education and Research Hospital, Department of Pathology, Malatya, Turkey
E-mail: bernaeriten@gmail.com

In the late 1980s, Holzmann B et al. proposed a model of melanoma in which benign melanocytes gradually evolved into melanoma cells with metastatic capability, in which every step was defined by the acquisition or loss of certain cellular markers that were easily detected by immunohistochemical analysis [4,5]. Of these, c-Kit (CD117) is a growth factor for melanocyte migration and proliferation. It shows different staining properties in various benign and malignant melanocytic lesions. While different rates of c-Kit expression were observed in some studies, the development of melanoma was found to be associated with loss of c-Kit expression in some studies [6,7].

Degradation of basement membranes and extracellular matrix is an essential step for melanoma cell migration, invasion, and metastasis formation in melanogenesis. Matrix metalloproteinases and their tissue inhibitors play a crucial role in these complex multistep processes. Melanoma cells may express a number of matrix metalloproteinase family members (MMP-1, MMP-2, MMP-9, MMP-13, and MT1-MMP) as well as their tissue inhibitors (TIMP-1, TIMP-2, and TIMP-3)[8]. The combination of determining MMP-2, Ki67, and p53 immunoreactive proteins could be beneficial in the selection of high-risk melanoma patients for future adjuvant trials. Increased expression and functionally

active of MMP-2 are associated with the progression of melanoma [9].

Ki-67, a determinant of proliferation kinetics, is not expressed in resting G0 cells of the cell cycle, but is expressed in G1, S, G2 and M phases. Therefore, the Ki-67 level is expected to be high in many aggressive MM cases [10-12].

Programmed cell death (apoptosis) has been implicated in tumor development and may affect the metastatic potential of tumor cells. The role of bcl-2, a proto-oncogene that inhibits apoptosis, has been studied in several malignancies, including cutaneous melanoma (CM). It is a family of pro- and anti-apoptotic proteins, regulates mitochondrial membrane permeability and proapoptotic mitochondrial pathway [13]. Dysregulation of Bcl-2 proteins appears of critical importance for melanoma cell survival and drug resistance, and among the fast increasing number of new targeted therapies, those, which affect Bcl-2 proteins, may especially apply for melanoma [14,15].

Metallothioneins (MT) are intracellular, low molecular weight, cysteine rich proteins. Its overexpression, which can be seen in many tumors, indicates resistance to anticancer drugs and radiotherapy and poor prognosis. A similar situation is also present in melanoma and non-melanoma skin tumors[16-18].

In our study, the relationship of these markers with selected prognostic factors (histological type, Breslow thickness, mitotic index, ulceration and localization) in cutaneous and mucosal malignant melanomas was investigated.

Materials and Methods

Ethics statement

Ethical approval for the study was obtained from Sivas Cumhuriyet University Scientific Research Assessment Board dated 04.12.2012 and numbered 2012-12/13. Institutional Review Boards of all participating institutions approved the study protocols and written informed consent was obtained from all study participants. Research methods of procedures were carried out in accordance with relevant guidelines and regulations.

30 CMM and 15 MMM cases diagnosed in SCU-TF-PD between 01 January 2000 and 01 June 2011 were included in the study. C-Kit, MMP-2, Ki-67, Bcl-2 and MT markers were applied to these cases. The relationship of these markers with selected prognostic factors (histological type, Breslow thickness, mitotic index, ulceration and localization) was investigated.

All H&E sections of FFPE tissues were removed from the archive. These were re-examined and sections containing melanoma foci suitable for research were determined. FFPE blocks belonging to the selected sections were detected. From these paraffin blocks, 3 µm thick sections were cut on slides covered with Poly-L-Lysine. Sections (Strept-) Avidin-Biotin-peroxidase (ABC-technique) with MMP-2 (Abcam, Mouse Monoclonal Antibody, clone 4D3, 1:20 dilution), bcl-2 (Novocastra, Mouse Monoclonal Antibody bcl-2 Oncoprotein , clone bcl-2/100/D5, ready-to-use form), MT (Invitrogen, Mouse Monoclonal Antibody, clone E9, ready-to-use form), Ki-67 (Biocare, Rabbit Monoclonal Antibody, clone SP6, ready-to-use form), c -Kit (Biogenex, Mouse Monoclonal Antibody, clone T595, ready-to-use form) immunohistochemistry dyes were applied [19].

Evaluation

Histological evaluation was performed by re-examining all H&E stained preparations of the cases, NICON, OPTIPHOT, JAPAN under the light microscope.

Information on the localization of the tumor was obtained from the SCU-TF-PD report archive.

Histological type was specified as NM, SSM, ALM, LMM and other types.

Breslow thickness was classified as ≤1mm, 1.01–2.0mm, 2.01–4.0 mm and> 4mm under the same microscope.

The mitotic index was counted in the "hot area" where the mitotic activity was the highest, that is, in 4x400 area (in mm²) in our microscope and grouped as 0, (0.1–6) and 6.

It was stated that the ulcer was present or absent.

External control preparations were prepared for each IHC staining.

MMP-2 expression was in the form of cytoplasmic staining and it was evaluated as negative if there is less than 5% staining, weak positive if there is 5-20%, moderate positive if there is staining in 21-50% malignant cells and strong positive if there is>50% staining [8].

Ki-67 expression was counted in the area of highest staining, given as a percentage. If staining <20%, it was considered as low expression, if ≥20% as overexpression [9].

Bcl-2 expression was also in the form of cytoplasmic staining, and staining less than 5% in tumor cells was accepted as 0 (negative), between 5-50% staining 1 (weak positive), and more than 50% staining 2 (strongly positive) [12].

MT expression is in the form of both cytoplasmic and nuclear staining. <10% staining in tumoral cells was considered negative, if there was more than 10% staining, it was accepted as overexpression. To prevent false positive results, 10% was taken as the cut-off value[14-16].

Cytoplasmic and membranous staining was sought for c-Kit expression. It was graded as 0 (negative) if there is no staining, if there is <10% (+), if there is 10-50% staining (++), if there is> 50% staining (+++) [6].

Results

The ages of the individuals in the CMM group varied between (3-89), with a mean (67.68±16.17), and the mean age (49-88) of the individuals in the MMM group (69.84±12.44). The difference between the two groups in terms of age was found to be insignificant (x^2:0.41; p:0.681;p>0.05).

Again, 17 (57%) of the individuals in the CMM group are male,

13(43%) are female; 7(47%) of the individuals in the MMM group were male and 8 (53%) were female, and the difference between groups in terms of gender was found to be insignificant (x^2:0.43; p:0.508; p>0.05).

Histological type

In CMMs, 22 cases (73.3%) were NM, 4 cases (13.3%) were SSM, 3 cases (10.0%) were LMM and 1 case (3.3%) was ALM. In MMM, 14 cases (93.3%) were in the NM type and 1 case (6.7%) was in the SSM type. When these groups were compared in terms of histological type, the difference between them was found to be insignificant (p> 0.05).

Breslow Thickness

7 cases (23.3%) with Breslow thickness ≤ 1.0 mm in CMMs, 4 cases (13.3%) with 1.01-2.0 mm, 4 cases (13.3%) with 2.01-4.0 mm and 15 cases with> 4 mm (50%). In MMMs, there were 1 case (6.7%) each with ≤ 1.0 mm and 1.01-2.0 mm, 5 cases (33.3%) with 2.01-4.0 mm and 8 cases (53.3%) with> 4 mm. When CMM and MMMs were compared in terms of Breslow thickness, the difference between the groups was found to be statistically insignificant (p>0.05).

Ulceration

While no ulcer was found in 13 cases (43.3%) in CMMs, ulcers were observed in 17 cases (56.7%). While there were no ulcers in 5 cases (33.3%) in MMMs, ulcers were observed in 10 cases (66.7%). When CMM and MMM were compared in terms of ulcer, the difference between the groups was found to be statistically insignificant (p>0.05).

Mitosis

13 cases (43.3%) without mitosis in CMMs, 15 cases (50%) with 0.1-6 mitosis and 2 cases (6.7%) with>6 mitosis were detected. There were 4 cases (26.7%) without mitosis in MMMs, 9 cases (60%) with 0.1-6 mitosis, and 2 cases (13.3%) with> 6 mitosis. When CMM and MMMs were compared in terms of mitosis, the difference between the groups was found to be statistically insignificant (p>0.05).

Localization: 11 (36.7%) of the CMMs were localized on the head and neck, 10(33.3%) on the extremities (hands and feet), and 9 (30.0%) on the trunk. 4(26.6%) of MMMs were conjunctiva, 4 (26.6%) were nasal passage, 2(13.3%) were vagina, 2 (13.3%) were oral cavities, 1 each was intestine (%) 6.6), vulva (6.6%) and cervix (6.6%).

Ki-67 index

There were 19 cases (65.5%) with Ki-67 index <20% and 10 cases (34.5%) with ≥20% in CMMs. (In this group, Ki-67 stain could not be applied in one case due to technical reasons and it was excluded from evaluation.) In MMMs, there were 5 cases (33.3%) with Ki-67 index <20% and 10 cases (66.7%) with ≥20%. When CMM and MMM groups were compared in terms of Ki-67 index, the difference was found statistically significant (p <0.05) (see in Table 1).

MMP-2 Expression

7 cases (24.1%) with <5% MMP-2 expression in their CMMs, 6 cases (20.7%) with 5-20%, 4 cases (13.8%) with 21-50% and 12 cases with >50% (% 41.4) was determined. (MMP-2 staining could not be applied in one case due to technical reasons and it was excluded from the evaluation.). There were no cases with MMP-2 expression rate <5% and 5-20% in MMMs. 7 cases (46.7%) with 21-50% staining and 8 cases (53.3%) with >50% staining were identified. When compared in terms of MMP-2 expression, the difference between groups was found to be statistically significant (p <0.05) (see in Table 2).

Table 1. Comparison of CMM and MMM in terms of Ki-67 index

		Ki-67		Total
		<%20	≥%20	
CMM	19	10	29	
	65.5	34.5	100.0	29
MMM	5	10	15	100.0
	33.3	66.7	100.0	15
Total	24	20	44	100.0
	54.5	45.5	100.0	44

Table 2. Comparison of CMM and MMM in terms of MMP-2 expression

		MMP-2				Total
		<% 5	% 5- 20	% 21- 50	>% 50	
CMM	N	7	6	4	12	29
	%	24.1	20.7	13.8	41.4	100.0
MMM	N	0	0	7	8	15
	%	0	0	46.7	53.3	100.0
Total	N	7	6	11	20	44
	%	15.9	13.6	25.0	45.5	100.0

X =11.3, p=0.010, p<0.05

bcl-2 Expression

10 cases (33.3%) with <5% staining in their CMMs, 6 cases (20.0%) with 5-50% staining and 14 cases (46.7%) with >50% expression were determined. Among MMMs, 6 cases (40.0%) with <5% staining and 9 cases (60.0%) with 50% staining were determined. There were no cases showing 5-50% expression in MMMs. When CMM and MMMs were compared in terms of bcl-2 expression, the difference was found to be statistically insignificant (p>0.05).

MT Expression

23 cases (76.7%) with <10% staining in their CMMs, 7 cases (23.3%) with 10% staining; 9 cases (60.0%) with <10% staining in MMMs and 6 cases (40.0%) showing >%10 staining were detected. When compared in terms of MT expression, the difference between groups was found to be statistically insignificant (p>0.05).

c-Kit Expression

No staining was detected in 8 (27.6%) of CMMs, <10% in 9 cases (31.0%), 10-50% in 7 cases (24%), and in 5 cases (17.2%) >%50 staining was seen at 50 percent. (Due to technical reasons, c-Kit staining could not be applied in one case and was excluded from the evaluation.) While no staining was detected in 3 (20.0%) of MMMs, <10% in 2 (13.3%) and 6 (40.0%) of them %10-50 and 4 (26.7%) of them >50% staining was observed. When CMMs and MMMs were compared in terms of c-Kit expression, the difference was found to be statistically insignificant (p>0.05).

Furthermore, when CMM and MMMs were compared in terms of Ki-67 expression according to their histological types, the difference between CMM cases was statistically significant (p <0.05), and the difference between MMMs was found insignificant (p> 0.05). In the CMM group, in NM cases, 15 cases (.68.2%) with a rate of <20% were detected. In non-skin MM, in NM cases, 10 cases (71.4%) with ≥20% staining were observed (see in Table. 3).

Table 3. Comparison of CMM and MMMs according to their histological types in terms of Ki-67 expression

Groups				Ki-67		Total
				<% 20	≥% 20	
CMM	Histological Type	NM	N	15	7	22
			%	68.2	31.8	100.0
		SSM	N	0	3	3
			%	0	100.0	100.0
		ALM	N	1	0	1
			%	100.0	0	100.0
		LMM	N	3	0	3
			%	100.0	0	100.0
	Total		N	19	10	29
			%	65.5	34.5	100.0
MMM	Histological Type	NM	N	4	10	14
			%	28.6	71.4	100.0
		SSM	N	1	0	1
			%	100.0	0	100.0
	Total		N	5	10	15
			%	33.3	66.7	100.0

X D=7.87, p=0.049, P<0.05
X DD=2.14, p=0.143 , P>0.05

When CMM and MMMs were compared in terms of MMP-2 expression according to their histological types, the difference was found to be statistically insignificant (p>0.05). However, in 10 (45.5%) of the NM's in the CMM group, MMP-2 expression was detected at a rate of >50%. MMP-2 expression was >50% in 8 (57.1%) of the NM in the MMM group (see in Table 4).

Table 4. Comparison of CMM and MMMs according to their histological types in terms of MMP-2 expression

Groups				MMP-2				Total
				<%5	%5- 20	%21-50	>%50	
CMM	Histological Type	NM	N	5	5	2	10	22
			%	22.7	22.7	9.1	45.5	100.0
		SSM	N	0	0	1	2	3
			%	0	0	33.3	66.7	100.0
		ALM	N	1	0	0	0	1
			%	100.0	0	0	0	100.0
		LMM	N	1	1	1	0	3
			%	33.3	33.3	33.3	0	100.0
	Total		N	7	6	4	12	29
			%	24.1	20.7	13.8	41.4	10.0
MMM	Histological Type	NMM	N			6	8	14
			%			42.9	57.1	100.0
		SSM	N			1	0	1
			%			100.0	0	100.0
	Total		N			7	8	15
			%			46.7	53.3	100.0

X D=8.69, p=0.466, P>0.05
X DD=1.22, p=0.268 , P>0.05

When CMM and MMMs according to histological types were compared in terms of bcl-2 expression, the difference was found to be statistically insignificant (p>0.05). However, bcl-2 expression was >50% in 10 (45.5%) of the NMs in the CMM group, while bcl-2 expression was >50% in 8 (57.1%) of the NMs in the MMM group (see in Table 5).

When CMM and MMMs according to histological types were compared in terms of MT expression, the difference was found to be statistically insignificant (p>0.05). However, while there were 17 cases (77.3%) with MT expression <10% in NM in the CMM group, 8 cases (57.1%) with MT expression <10% in NM in the MMM group were detected (see in Table 6).

When CMM and MMMs were compared in terms of c-Kit expression according to histological types, the difference was found to be statistically insignificant (p>0.05). On the other hand, 8 cases (36.4%) were detected with <10% staining in NM in the CMM group, and 10-50% was observed in 5 cases (35.7%) of the NM type in MMM (see in Table 7).

Table 5. Comparison of bcl-2 expression of CMM and MMM according to histological types

Groups				bcl-2				Total
				<%5	%5-50	>%50	>%50	
CMM	Histological Type	NM	N	9	3	10	22	22
			%	40.9	13.6	45.5	100.0	100.0
		SSM	N	1	2	1	4	3
			%	25.0	50.0	25.0	100.0	100.0
		ALM	N	0	0	1	1	1
			%	0	0	100.0	100.0	100.0
		LMM	N	0	1	2	3	3
			%	0	33.3	66.7	100.0	100.0
	Total		N	10	6	14	30	29
			%	33.3	20.0	46.7	100.0	10.0
MMM	Histological Type	NM	N	6	0	8	14	14
			%	42.9	0	57.1	100.0	100.0
		SSM	N	0	0	1	1	1
			%	0	0	100.0	100.0	100.0
	Total		N	6	0	9	15	15
			%	40.0	0	60.0	100.0	100.0

X D=5.78, p=0.448, P>0.05
X DD=0,71, p=0.398, P>0.05

Table 6. Comparison of CMM and MMMs in terms of MT expression according to their histological types

Groups				MT		Total
				<%10	>%10	
CMM	Histological Type	NM	N	17	5	22
			%	77.3	22.7	100.0
		SSM	N	2	2	4
			%	50.0	50.0	100.0
		ALM	N	1	0	1
			%	100.0	0	100.0
		LMM	N	3	0	3
			%	100.0	0	10.0
	Total		N	23	7	30
			%	76.7	23.3	100.0
MMM	Histological Type	NM	N	8	6	14
			%	57.1	42.9	100.0
		SSM	N	1	0	1
			%	100.0	0	100.0
	Total		N	9	6	15
			%	60.0	40.0	100.0

X D=2.81, p=0.422, P>0.05
X DD=0,71, p=0.398, P>0.05

Table 7. Comparison of CMM and MMM according to histological types in terms of c-Kit expression

Groups				c-Kit				Total
				0	< %10	% 10-50	>% 50	
CMM	Histological Type	NM	N	7	8	3	4	22
			%	31.8	36.4	13.6	18.2	100.0
		SSM	N	0	1	1	1	3
			%	0	33.3	33.3	33.3	100.0
		ALM	N	1	0	0	0	1
			%	10..0	0	0	0	100.0
		LMM	N	0	0	3	0	3
			%	0	0	100.0	0	100.0
	Total		N	8	9	7	5	29
			%	27.6	31.0	24.1	17.2	100.0
MMM	Histological Type	NMM	N	3	2	5	4	14
			%	21.4	14.3	35.7	28.6	100.0
		SSM	N	0	0	1	0	1
			%	0	0	100.0	0	100.0
	Total		N	3	2	6	4	15
			%	20.0	13.3	40.0	26.7	100.0

X D=14.80, p=0.097, P>0.05
X DD=1.60, p=0.658 , P>0.05

Discussion

The frequency of MM among all skin cancers is 3-5%. It is responsible for 75% of deaths due to skin cancers and 1-2% of deaths due to all cancers [1-3, 20]. It is stated that the incidence of whites in industrial countries has increased significantly in the last two decades, and nowadays its incidence has stabilized and even decreased. The incidence of melanomas is highest in Northern Australia. There are 42.89 new cases per 100.000 in women and 55.8 new cases in men every year. This rate is 8 in England, 24.4 in South Africa and 4.9 in Scotland. While the lifetime risk of melanoma development was 1 in 120 in the USA in 1987, this rate was reported as 1 in 75 in 2000 [21].

In a study conducted in Germany, the number of cases documented annually increased by 53.2% between 2002 (N=4779) and 2011 (N=7320). There was a statistically significant continuous positive trend in the proportion of stage UICC I cases diagnosed between 2002 and 2011 compared with a negative trend for stage UICC II. No trends were found for UICC III and IV stages, respectively [22].

Survival rates increase in patients with melanoma, as patients present earlier and with thinner melanoma. Five-year survival rates have been reported at 85% or more in women and 75% in men. Again, in another cohort analysis of 4791 patients diagnosed with primary CMM between 1976 and 2001 in southern Germany, primary CMM diagnosis during 1990-2001 was associated with a more favorable 10-year survival (88.6% versus 80.0%, P<.0001) compared with 1976-1989. Median tumor thickness at primary diagnosis was significantly lower in the second period (0.75mm vs

1.07 mm, P<.0001). That is, tumor thickness has been found to be a dominant prognosis determinant [23].

In the study, the number of patients with ulcers (66%) and the number of cases with Breslow thickness >4 mm (53%) were higher in MMMs compared to CMMs. This situation may be associated with the worse prognosis in MMMs compared to skin melanomas.

In MMMs, there are many prognostic factors other than tumor thickness that have different importance. These are histological type, ulceration, mitotic index, presence of satellites, angiolymphatic invasion, advanced stage, occult metastases, local recurrence, Clark invasion depth, location, accompanying nevus, lymphocytic infiltration, regression, nuclear volume, sex, vitiligo, age and can be counted as pregnancy [1-3, 24].

According to AJCC staging, three important histopathological criteria are Breslow thickness, ulcer and mitotic index [25]. Age, gender, and anatomic location of the primary tumor that are not included in the AJCC system also affect the survival of primary MM cases [26,27]. When these factors are combined with histopathological features, they show a better prognosis than AJCC system [24].

Gonzalez et al. showed that melanoma expresses c-kit, a gastrointestinal stromal tumor marker, but has not been extensively evaluated for protein kinase Cθ (PKCθ) or DOG1, and these stains have not been associated with prognostic factors. They immunostained 62 primary cutaneous and 15 metastatic melanomas for polyclonal c-kit (pc-kit), monoclonal c-kit (mc-kit), PKCθ, and DOG1, and correlated the results with prognostic parameters and survival. 34 (55%) of cutaneous melanomas were stained for pc-kit, 30 (48%) for mc-kit, 11 (18%) for PKCθ, and 2 (3%) for DOG1 [28].

Liu et al. investigated c-Kit and Sox10 expressions in 28 patients with sinonasal mucosal melanoma, and determined c-Kit expression in 24 patients, therefore they emphasized that c-Kit expression may be useful in the regulation of treatment. However, they also stated that Sox10 is a sensitive determinant in SSMs [29]. In the study, c-Kit expression was detected in all conjunctival MM's. In addition, CK-17 expression was found in all but 3 of 11 nonconjunctival cases in the MMM group.

Weinlich et al. recently investigated the role of MT overexpression in sentinel lymph node biopsies in melanoma progression in 158 cases in 2007, and determined that metastases developed in 28 patients (17.7%), and 17(10.7%) died from disseminated disease; demonstrated that the results support the validity of MT overexpression as a useful prognostic marker in patients with primary melanoma [18]. In the study, the number of cases with MT expression <10% was found to be higher in the skin and extradermal group than the cases with MT overexpression. However, most of the MT negative cases were histologically of NM type and Breslow thickness was higher. In addition, in the MMM group, in patients with low MT expression, ulcers were observed at a higher rate. These findings oft he study can be interpreted as indirectly, that the loss of MT expression indicates a serious prognosis, albeit a little.

Espindola and Corleta detected 74.3%, 85.7%, and 82.4% bcl-2 expression in lymph node, subcutaneous and visceral metastases, respectively, but after univariate and multivariate analyzes, positive bcl-2 expression and overall survival for the types of metastases evaluated were no correlation was found [13]. In our series, bcl-2 expression among CMMs, 10 cases (33.3%) with <5% staining, 6 cases (20.0%) with 5-50% staining and 14 cases (46.7%) with >50% expression was found. Also, among MMMs, 6 cases (40.0%) with <5% staining and 9 cases (60.0%) with 50% staining were determined. There were no cases showing 5-50% expression in MMMs. When CMM and MMMs were compared in terms of bcl-2 expression, the difference was found to be statistically insignificant (p>0.05).

The tumor stage is the most critical factor in determining prognosis in cutaneous and mucosal malignant melanomas tumors. Non-skin malignant melanoma carcinoma has a favorable prognosis if it is not associated with carcinoma in situ or upper system transitional cell carcinoma. However, while progression is uncommon in these patients, it is possible. It is extremely difficult to predict which cases will progress and when they will progress. The proliferation index Ki-67 and MMP-2 overexpression appear to be significant in predicting the progression of non-skin malignant melanoma carcinoma. The proportion of Ki-67 positive cells in low-grade tumors was determined immunohistochemically in studies using Ki-67, and similar results were obtained [30]. The correlation between the Ki-67 index and progression has been demonstrated in cancers that do not invade muscle [31,32,33]. Given that rapidly dividing tumors frequently progress aggressively, the positive correlation between the Ki-67 proliferation index and progression is unsurprising, as Ki-67 is one of the immunohistochemical markers capable of predicting progression. They stated that, in contrast to our study, the relevant literature indicates that IHC can be used as a biomarker that predicts progression and survival by combining multiple methods, not just one. To corroborate our findings, numerous studies have demonstrated an association between MMP-2 and grade [34-37].

Conclusion

As a result, when the CMM and MMM groups were compared, the difference between them was statistically significant in staining with Ki-67 and MMP-2, while staining with c-Kit, MT and bcl-2 was statistically insignificant. It was thought that high MMP-2, c-Kit, bcl-2 expressions and high Ki-67 index as well as MT expression loss were thought to be associated with poor prognosis when evaluated with associated histological prognostic factors. However, in order to precisely determine the prognosis of MMs and to determine more effective treatments, larger case series should be followed with molecular pathology studies.

Conflict of interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical approval
Ethical approval for the study was obtained from Sivas Cumhuriyet University Scientific Research Assessment Board dated 04.12.2012 and numbered 2012-12/13.

References

1. Kumar V, Abbas AK, Fausto N, et al. Robbins and Cotran pathologic basis of disease, professional edition e-book. Elsevier Health Sciences,

Philadelphia, 2014.

2. Mooi W, Krausz T. Pathology of melanocytic disorders. 2nd edition. CRC Press Italy. 2007;2:496.

3. Weedon D. Lentigines, nevi, and melanomas. In: Chapter32: Lentigines, Nevi and Melanomas. Weedon's Skin Pathology. Churchill Livingstone Elsevier British. 2010;734-54.

4. Fernandez-Flores A. Prognostic factors for melanoma progression and metastasis: from Hematoxylin-Eosin to genetics. Rom J Morphol Embryology. 2012;53:449-59.

5. Holzmann B, Bröcker EB, Lehmann JM, et al. Tumor progression in human malignant melanoma: five stages defined by their antigenic phenotypes. Int J Cancer. 1987;39:466–471.

6. Pilloni L, Bianco P, Difelice E et al. The usefulness of c-Kit in the immunohistochemical assessment of melanocytic lesions. Eur J Histochem: 2011;55:20.

7. Potti A, Hille RC, Koch M. Immunohistochemical determination of HER-2/neu overexpression in malignant melanoma reveals no prognostic value, while c-Kit (CD117) overexpression exhibits potential therapeutic implications. J Carcinog. 2003;2:8.

8. Hofmann UB, Westphal JR, Van Muijen GN, et al. Metalloproteinases in human melanoma. J Invest Dermatol. 2000;115:337-44.

9. Väisänen A, Kuvaja P, Kallioinen M, et al. A prognostic index in skin melanoma through the combination of matrix metalloproteinase-2, Ki67, and p53. Hum Pathol. 2011;42:1103-11.

10. Hazan C, Melzer K, Panageas KS, et al. Evaluation of the proliferation marker MIB-1 in the prognosis of cutaneous malignant melanoma. Cancer. 2002;95:634-40.

11. Tu TJ, Ma MW, Monni S, et al. A high proliferative index of recurrent melanoma is associated with worse survival. Oncology. 2011;80:181-7.

12. Ladstein RG, Bachmann .M, Straume O, et al. Ki-67 expression is superior to mitotic count and novel proliferation markers PHH3, MCM4 and mitosin as a prognostic factor in thick cutaneous melanoma. BMC Cancer. 2010;10:140.

13. Espíndola MB, Corleta OC. bcl-2 expression is not associated with survival in metastatic cutaneous melanoma: a historical cohort study. World J Surg Oncol. 2008;6:65.

14. Eberle J, Hossini AM. Expression and function of bcl-2 proteins in melanoma. Curr Genomics. 2008;9:409-19.

15. Pisano M, Baldinu P, Sini MC, et al. Targeting bcl-2 protein in treatment of melanoma still requires further clarifications. Ann Oncol. 2008;19:2092-3.

16. Weinlich G, Bitterlich W, Mayr V, et al. Metallothionein-overexpression as a prognostic factor for progression and survival in melanoma. A prospective study on 520 patients. Br J Dermatology. 2003;149(3):535-541.

17. Weinlich G, Eisendle K, Hassler E, et al. Metallothionein-overexpression as a highly significant prognostic factor in melanoma: a prospective study on 1270 patients. Br J Cancer. 2006;94:835-41.

18. Weinlich G, & Zelger B. Metallothionein overexpression, a highly significant prognostic factor in thin melanoma. Histopathology. 2007;51:280-3.

19. Jackson P, Blythe D. Immunohistochemical techniques. Theory and Oractice of Histological Techniques. Churchill Livingstone Elsevier, British, 2008;6:440-1.

20. Shay JW. New insights into melanoma development. Science. 2017;357:1358-9.

21. Shenenberger, DW. Cutaneous malignant melanoma: a primary care perspective. Am Fam Physicianology. 2012;85:161-8.

22. Schoffer O, Schülein S, Arand G, et al. Tumour stage distribution and survival of malignant melanoma in Germany 2002-2011. BMC Cancer. 2016;16:936.

23. Lasithiotakis KG., Leiter U, Eigentler T, et al. Improvement of overall survival of patients with cutaneous melanoma in Germany, 1976-2001: which factors contributed? Cancer. 2007;109:1174-82.

24. Mervic L. Prognostic factors in patients with localized primary cutaneous melanoma. Acta Dermatovenerol Alp Pannonica Adriat. 2012;21:27-31.

25. Balch CM, Gershenwald JE, Soong SJ, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol. 2009;27:6199.

26. Gershenwald JE, Soong SJ, Balch CM, et al. 2010 TNM staging system for cutaneous melanoma… and beyond. Ann Surg Oncol. 2010;17:1475-7.

27. Nading MA, Balch CM, Sober AJ. Implications of the 2009 American Joint Committee on Cancer Melanoma Staging and Classification on dermatologists and their patients. Semin Cutan Med Surg. 2010;29:142-7.

28. Gonzalez RS, Carlson G, Page AJ, et al. Gastrointestinal stromal tumor markers in cutaneous melanomas: relationship to prognostic factors and outcome. Am J Clin Pathol. 2011;136:74-80.

29. Liu HG, Kong MX, Yao Q, et al. Expression of Sox10 and c-kit in sinonasal mucosal melanomas arising in the Chinese population. Head Neck Pathol. 2012;6:401-8.

30. Wang G, Black PC, Goebell PJ, et al. Prognostic markers in pT3 bladder cancer: A study from the international bladder cancer tissue microarray project. Urol Oncol. 2021;39:301-e17.

31. Parizi MK, Margulis V, Lotan Y, et al. Fibroblast growth factor receptor: a systematic review and meta-analysis of prognostic value and therapeutic options in patients with urothelial bladder carcinoma. Urol Oncol. 2021; 39:409-21.

32. Jäälinojä, J, Herva R, Korpela M, et al. Matrix metalloproteinase 2 (MMP-2) immunoreactive protein is associated with poor grade and survival in brain neoplasms. J Neurooncol. 2000;46:81-90.

33. Vafadari B, Salamian A, Kaczmarek L. MMP-9 in translation: from molecule to brain physiology, pathology, and therapy. J Neurochem. 2016; 139:91-114.

34. Turpeenniemi-Hujanen T. Gelatinases (MMP-2 and -9) and their natural inhibitors as prognostic indicators in solid cancers. Biochimie. 2005; 87:287-97.

35. Yan S, Xue H, Zhang P, et al. MMP inhibitor Ilomastat induced amoeboid-like motility via activation of the Rho signaling pathway in glioblastoma cells. Tumour Biol. 2016; 37:16177-86.

36. Li Q, Chen B, Cai J, et al. Comparative Analysis of Matrix Metalloproteinase Family Members Reveals That MMP9 Predicts Survival and Response to Temozolomide in Patients with Primary Glioblastoma. PLoS One. 2016; 11:e0151815.

37. Joseph JV, Roosmalen IAV, Busschers E, et al. Serum-Induced Differentiation of Glioblastoma Neurospheres Leads to Enhanced Migration/Invasion Capacity That Is Associated with Increased MMP9. PLoS One. 2015;1:e0145393.