NOWOTWORY Journal of Oncology • 2002 • volume 52
Number 6• 487–495

Prognostic value of selected immunohistochemical markers in skin melanoma

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Aim: to assess the significance of the expression of metalloproteinase 2 (MMP-2), CD44 glycoprotein and nm23 diphosphate nucleoside kinase on melanoma progression and survival time and its comparison with the influence of clinical and histopathological parameters.
Material and metods: 39 patients operated for the first time because of melanoma in the 2nd Department of Oncological Surgery of the Greatpoland Cancer Centre in Poznan between the years 1990-1994.
Clinical data such as age, gender, the occurrence of cancer spread, its location and survival time were assessed.
Using archival histopathological specimens we assessed the thickness of tumour invasion in mm according to Breslow, the level of invasion according to Clark, histological type, the presence of lymphocyte infiltration, regression symptoms, the existence of preexisting naevus, the presence of ulceration, the presence of satellites and metastases to lymphatic nodes.
Immunohistochemical marker expression was performed in tissue material obtained from archival paraffin blocks. The presence of HMB-45 and PCNA, the expression of metalloproteinase 2 (MMP-2), CD44 glycoprotein and nm23 diphosphate nucleoside kinase were also assessed. Clinical, histopathological and immunohistochemical data was analysed in correlation with survival time. The evaluation of prognostic factors was performed by Cox's nonparametric proportional hazard regression model.
Results: the crucial prognostic factor in the examined group turned out to be the presence of a positive reaction product at the site of nm23 diphosphate nucleoside kinase existence in correlation with both clinical and histological factors. In the grow-up of pathological factors the prognostic significance of the lymphatic metastases at the onset of treatment was demonstrated.
Conclusions: the results indicate that the greatest prognostic value of skin melanoma progression lies in the demonstration of nm23 diphosphate nucleoside kinase in skin.

Markery immunohistochemiczne w czerniaku

Cel pracy. Celem pracy jest ocena znaczenia metaloproteinazy 2 (MMP-2), glikoproteiny CD44 i nm23 kinazy dwufosfonukleozydowej dla przebiegu czerniaka skóry w porównaniu z wpływem czynników klinicznych i histopatologicznych na czas przeżycia.
Materiał i metodyka. Analizowano dane 39 pacjentów operowanych z powodu czerniaka w Wielkopolskim Centrum Onkologii w Poznaniu w II Oddziale Chirurgii Onkologicznej po raz pierwszy w latach 1990-1994. Ocenie poddano dane kliniczne, takie jak: wiek, płeć, pojawienie się rozsiewu nowotworowego, jego lokalizację oraz czas przeżycia.
Na podstawie archiwalnych preparatów histopatologicznych oceniono: grubość nacieku guza w mm wg Breslow'a, poziom naciekania wg Clarka, postać histologiczną, obecność nacieku limfocytarnego, cechy regresji, obecność owrzodzenia, obecność satelitów i przerzutów do węzłów chłonnych. Przeprowadzono badania ekspresji markerów immunohistochemicznych w materiale tkankowym uzyskanym z archiwalnych bloczków parafinowych. Określono obecność HMB-45 i PCNA, ekspresję metaloproteinazy 2 (MMP-2), glikoproteiny CD44 i nm23 kinazy dwufosfonukleozydowej. Dane kliniczne, histopatologiczne i immunohistochemiczne oceniono w korelacji z czasem przeżycia. Ocenę czynników prognostycznych przeprowadzono metodą nieparametrycznego modelu proporcjonalnego hazardu wg Coxa.
Wyniki. Najistotniejszym czynnikiem rokowniczym w badanej grupie okazał się dodatni wynik reakcji na obecność nm23 kinazy dwufosfonukleotyzydowej i to zarówno w korelacji z czynnikami klinicznymi jak i histopatologicznymi. Spośród czynników patoklinicznych stwierdzono znaczenie rokownicze obecności przerzutów w węzłach chłonnych w chwili rozpoczęcia leczenia.

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Wnioski. Przeprowadzone badanie wykazało w badanej grupie chorych przydatność oznaczania nm23 kinazy dwufosfonukleozydowej w prognozowaniu przebiegu czerniaka skóry.

Key words: malignant melanoma, prognostic factors, immunohistochemical markers
Słowa kluczowe: czerniak, czynniki prognostyczne, markery immunohistochemiczne

Introduction

The clinical diagnosis of melanocytic lesions suspected of skin melanoma usually arises from a change in the appearance of skin lesion on the basis of characteristic symptoms classified in two systems: ABCD and the 7--point Glasgow scale. Some lesions with no clear-cut clinical diagnosis can be qualified for excision biopsy leaving a healthy skin margin, also broadened by a suitable fragment of subcutaneous lipid tissue to perform an exact histological assessment [1].

Morphological details of melanoma also allow for a preliminary assessment of progression and for establishing a treatment course.

Typical histological prognostic factors are: the thickness of melanoma measured by Breslow method in mm [2], the status of regional lymphathic nodes [3-6], the level of invasion measured according to Clark [2-7], histological type of Superficial Spreading Melanoma Malignum (SSMM) [7-11] (with better prognosis), ulceration and vessel invasion (which are closely related to tumour invasion level and do not remain independent prognostic factors) [9], and lymphocyte infiltration (examined by Clemente et al.) [5].

The most frequently considered clinical parameters are: age (Balzi et al. [8] estimated the age above 60 to have a better prognosis; according to Kuno et al. the limit is the age of 70 [12]), male sex and tumour location on the trunk, shoulder girdle, neck or head [5, 8, 10, 13].

The only independent prognostic factors according to many authors [3, 7, 9, 11] are lesion thickness, the existence of lymphatic metastases detected at the beginning of treatment and the degree of clinical advance estimated on that basis.

The search for, and assessment of, more prognostic factors is indispensable to improve the results of treatment.

The most frequently assessed immunohistochemical markers are: S-100 protein, HMB-45, vimentin, tirosinase (TRP-1) and proliferative factors such as p53 protein, Ki-67 (MIB1) and PCNA. According to the results of Niezabitowski et al. another independent prognostic factor was proliferative antigen expression (PCNA and Ki 67 [5]). Other authors emphasize the importance of PCNA and Ki67 in the differentiation of melanoma cells, its proliferative abilities and indirect influence on tumour invasion level, however they do not always confirm their correlation with survival probability [14-16]. Karbowniczek et al. [14] have described an association between the area and shape of melanoma cell nuclei (with positive results for Ki67) and the presence of metastases, and between the nuclear area and of tumour cells and such factors related to poor prognosis as the depth of invasion and tumour thickness. Talve et al. [15] have stressed the importance of proliferative parameters such as Ki67 and mitotic index, however they did not prove their influence on the predicted survival rate. Rudolph et al. [16] have proven the value of Ki67 for the diagnosis and differentiation of benign melanocytic changes from melanoma. Other markers, such as HMB-45, S-100, vimentin and tirosinase, have been applied in the diagnosis and differentiation research but did not display any prognostic value.

However a group of new melanoma markers including metalloproteinase-2 (MMP-2), CD44 glycoprotein and nm23 diphosphate nucleoside kinase has been discerned.

Metalloproteinases (MMP) are a group of enzymes responsible for rebuilding structural proteins of extracellular spaces and basement membrane. MMP consists of 17 enzymes belonging to different groups (collagenase, gelatinase, stromatin enzyme, membrane MMP and others). They take part in connective tissue transformations, stimulation of plate aggregation (regardless of A2 thromboxane), the process of wound healing, angiogenesis, the process of adhesion and cell migration through biological membranes. First of all MMP's are produced by monocytes, macrophages and granulocytes. They are also involved in tumor metabolism and are secreted by adenocarcinoma cells of the digestive tract, breast, stomach, liver, colon, prostate, ovary and cystic urinary cancers. The increase of MMP level is also related to the direct damage of tissue structure which causes the activation of the physiological regeneration processes, that is why MMP level is increased in inflammatory conditions, for example in osteoporosis, rheumatoid arthritis, chronic lung disease, hepatitis etc. Metalloproteinases are one of the elements of melanoma metabolism and they also have some influence on its growth, invasion and metastatic potential. Their activity on invasive melanoma cells along with the presence of superficial glycoprotein CD44 and integrin alphavbeta3 [17 18] was confirmed. In 1985 Väisänen et al. provided the first publication concerning the influence of collagenase IV, belonging to the group of gelatinases (gelatinase A, MMP-2), on the course of melanoma [19]. In this and in a number of further papers the importance of MMP-2 as a prognostic parameter of melanoma was proven [19, 20]. Direct significance of MMP-2 for tumour cell metabolism is based on increasing the abilities of biological membranes invasion and changing adhesive abilities, which makes them capable of migration and colonization, thus forming distant metastases. Direct connection was found between MMP-2 and the increased risk of distant metastases by blood and it

was shown to be a prognostic factor of survival time, regardless of the thickness of invasion according to Breslow and the level of invasion according to Clark. MMP--2 expression was related to the level of clinical advancement and patient gender. The higher survival rate in women than in men still remains unexplained. The connection between metastases occurring in women with breast cancer and hormonal activity was indicated. Oestrogens are influencing the regulation of collagen IV secretion, which reduces breast cancer cell activity. This leads to a reduction of metalloproteinase secretion (gelatinase B). According to Väisänen et al. there may also exist an influence of oestrogens on MMP-2 decreasing the risk of melanoma metastases by blood in women [19].

The prognostic value of proteolytic enzymes in melanoma was also studied by Otto et al. [21]. Differences in the occurrence of MMP-2 in patients suffering from melanoma with and without metastases were shown. Moreover, the significant prognostic parameters in the above--mentioned study were: positive reaction to the appearance of catepsine B and D, gender, lesion location, tumour thickness according to Breslow, the level of invasion according to Clark and the presence of ulceration.

During the research on melanoma immunohistochemical markers we also studied the elements of protein and receptor structure of melanoma cells and connective tissue, which come into contact with each other during migration and metastases formation. Significance is attributed to superficial CD44 receptors responsible for intercellular adhesion and for adhesion between melanoma cells and structural connective tissue (matrix) [22, 23]. It has been proven that CD44 glycoprotein expression on the surface of melanoma cells increases migration and invasion ability contributing to the increased risk of metastases and melanoma progression [22]. Thomas et al. [24], Goebeler et al. [25], Maaser et al. [26] and other authors [27, 28] have demonstrated increased migration ability in melanoma cells with the presence of antigen CD44 in an environment of hyaluronic acid. Takahashi et al. [29] has shown the connection of CD44 expression with MMP--2 in melanoma cells metabolism.

Numerous proteins are responsible for the complex process of melanoma metabolism, their expression is controlled by genes. One of the earlier studied suppressor genes located on the 17q chromosome is a gene responsible for nm23 diphosphate nucleoside kinase production. The assessment of this protein expression proved inhibition of melanoma progression [30-34]. Lee et al. [30], Betke et al. [31], Florens et al. [32] confirmed the significance of nm23 as a prognostic factor. Nm23 protein influences the degree of tumour differentiation and its proliferative abilities. However not all authors confirm its correlation with the formation of remote metastases and direct influence on survival time [34-38].

Malignant melanoma has a high malignancy and a surprising disease progression is sometimes noticed. Therefore much research goes on to attempt to explain this problem. The existence of numerous melanoma cell lines makes it difficult to search for universal and repeatable markers and prognostic factors. The assessment of an greater number of prognostic factors may make it easier to establish new standards of melanoma treatment.

The aim of the study

The aim of this study was to assess the significance of metalloproteinase 2 (MMP-2), CD44 glycoprotein and nm23 diphosphate nucleoside kinase on melanoma progression and survival time, as compared to the influence of clinical and histopathological parameters.

Material and methods

The clinical, histopathological and immunohistochemical data is presented in Tables I – III. We have performed an analysis of 39 patients operated for the first time for melanoma at the Greatpoland Cancer Centre between the years 1990-1994. Clinical data such as age, gender, the occurrence of tumor spread, its location and survival time were assessed. Histological assessment was performed on archival routine hematoxyline/eosin – stained histopathological specimens which were described in terms of the thickness of melanoma infiltration in mm acc. to Breslow, the level of invasion acc. to Clark, histological type, the presence of lymphocyte infiltration, regression features, the existence of a pre-existing naevus, the presence of ulceration and the the existence of satellites and lymphatic metastases.

Table I. Clinical characteristics of patients

Characteristic		No	Percentage
Number of patients		39	100 %
Mean age, range		51,35 (27-79)	
Sex	male	9	23.07 %
	female	30	76.92 %
Five year surviving		24	61.54 %
Dissemination		18	46.15 %
Site of metastases	lymph node	15	38.46 %
	skin	8	20.51 %
	internal organs	12	30.76 %
Lesion location	trunk, shoulder girdle, neck or head	17	43.58 %
	limbs	22	56.41 %
Range of first operation			
Lymphadenectomy carried out along with tumor excision		17	47.22 %
Tumour excision		19	52.78 %

Tests for immunohistochemical marker expression were performed on the material received from archival paraffin blocks containing tumor tissue, which had been 10% formalin fixed and embedded according to typical histological methods. 4-5μm sections were prepared. Amplification of reaction products was performed using the En Vision + ™ /HRP (Mouse and Rabbit) complex. The presence of HMB-45 and PCNA was determined. The expression of metalloproteinase 2 (MMP-2), CD44 glycoprotein and nm23 diphosphate nucleoside kinase from the group of lastest melanoma markers was examined. Positive and negative results of staining were analysed.

Survival analysis was performed using the Kaplan-Meier method. Statistical significance of prognostic factors depending on survival time was assessed by Cox nonparametric proportional hazard regression model.

Results

The average age of the patients in the examined group was 51,3 years old (27-79 years + 13.11 SD). The group consisted of 30 women (76.9%) and 9 men (23.1%). 24 patients (61.5%) survived over 5 years. Lesions originating from the trunk, head, neck, shoulder girdle were found in 17 patients (43.6%) and from the limbs in 22 patients (56.4%). Melanoma spread during observation was detected in 18 patients (46.2%). Metastases were most frequent in the lymphatic nodes – 15 cases (38.5%), in internal organs – 12 cases (30.8%) and in the skin – 8 cases (20.5%). As to the thickness of lesions according to Breslow, 6 patients (15.3%) had a Breslow score <0.75 mm; 10 patients (25.6%) – 0.76-1,5 mm; 8 patients (20.5%) – 1.51--4.0 mm; 15 patients (38.6%) ->4mm. Level II invasion according to Clark was found in 7 patients (17.9%), level III in 17 (43.6%), level IV in 12 (30.8%) and level V in 3 (7.7%). In the examined group of patients regional lymphatic metastases were found in 15 patients at the beginning of treatment. Superficial forms of SSMM type melanoma were found in 35 patients (87.2%), and of tumour type – in 5 patients (12.8%). Ulceration was found in 19 patients (48,7%). Regression symptoms were found in 19 patients. Lymphocyte infiltration occurred in 23 patients (58.97%). The existence of a preexisting naevus was found in 7 patients (17.9%). Amelanotic type melanomas were present in 12.82% of the examined group (5 patients). No satellite changes were found in the examined patients.

Suitable specimens for immunohistochemical studies were unavailable in 3 cases. Those 3 patients were excluded from further statistical analysis.

Table II. Histological characteristics of tumor data

Characteristic		No	Percentage
Histology type	SSMM	34	87.18 %
	NMM	5	12.82 %
Thickness	0 – 0.75	6	15.38 %
according to	0.76 – 1.5	10	25.64 %
Breslow	1.51 – 4.0	8	20.51 %
	>4.0	15	38.56 %
Level	I		
of invasion	II	7	17.95 %
according to	III	17	43.58 %
Clark	IV	12	30.76 %
	V	3	7.69 %
Metastases to lymph nodes		15	38.46 %
Lymphocyte	yes	23	58.97 %
infiltration	no	16	41.03 %
Regression	yes	19	48.71 %
symptoms	no	20	51.29 %
Presence of	yes	19	48.71 %
ulceration	no	20	51.29 %
Satellites	yes	0	0
	no	39	100 %
Preexisting naevus		7	17.94 %
Melanin	yes	34	87.18 %
pigment	no	5	12.82 %

Table III. Immunohistochemical data

Immunohistochemical factor		No	Percentage
HMB45	positive	36	100 %
	negative	0	0 %
	no result	0	0 %
PCNA	positive	24	66.7 %
	negative	12	33.3 %
	no result	0	0 %
nm23	positive	20	55.6 %
	negative	11	30.5 %
	no result	5	13.9 %
MMP-2	positive	13	36.1 %
	negative	22	61.1 %
	no result	1	2.8 %
CD44	positive	20	55.6 %
	negative	13	36.1 %
	no result	3	8.3 %

In some cases it was difficult to assess the expression of nm23, CD44 and MMP-2 owing to the presence of excess melanin, which masked immunohistological reactions. The interpretation of the results was based on comparison with hematoxyline/eosin-stained specimens.

We did not manage to assess markers for nm23 diphosphate nucleoside kinase in 5 patients (13.9%), for glycoproteinase CD44 in 3 cases (8.3%) and for metalloproteinase 2 (MMP-2) in 1 case (2.8%). PCNA and HMB-45 were marked in all cases.

The HMB-45 marker reacted positively in 36 patients (100%). PCNA marker reacted positively in 24 patients (66.7%) and negative reactions were observed in 12 (33.3%). MMP-2 expression was detected in 13 patients (36.1%) and negative reactions were observed in 22 (61.1%). Nm23 diphosphate nucleoside kinase was detected in 20 patients (55.6%) and negative reactions were observed in 11 (30.5%). The existence of CD44 glycoprotein receptors was reported in 20 patients (55.6%) and negative reactions were seen in 13 (36.1%). Positive reactions to the existence of immunohistochemical markers mentioned above are presented in Fig. 1-5.

The median survival time according to the Kaplan--Meier method was 86,4 months, lower quartile (25th percentile) was 28 months. The curve of survival function is presented in Fig. 6.

Statistical significance of the examined prognostic factors was assessed using Cox's proportional hazards regression model of survival.

Due to the lack of satellite changes and 100% positive reaction to HMB-45 antigen existence these indicators were excluded from statistical analysis in the examined patients.

The following parameters were analysed: age, gender, lesion location, the range of surgery (lymphadenectomy carried out along with tumour excision), the depth of invasion in mm according to Breslow, the level of invasion according to Clark, the existence of lymphatic metastases, histological type of SSMM, ulceration, regression factors, the presence of a preexisting naevus, the presence of

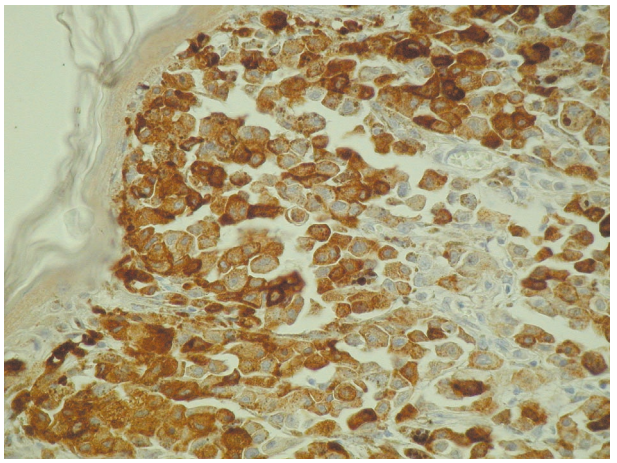

Figure 1. Positive staining: HMB-45 (400x)

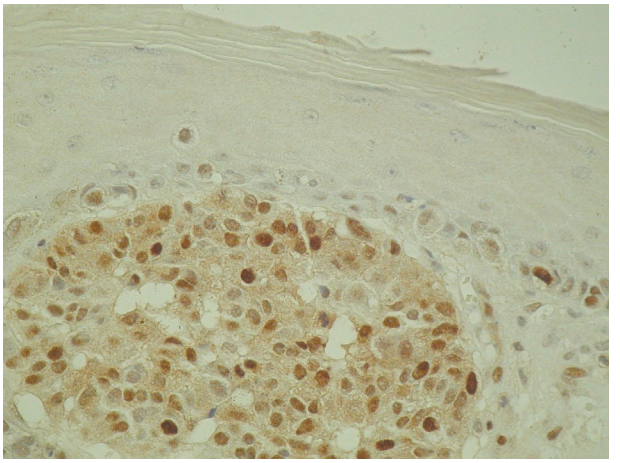

Figure 2. Positive staining PCNA (400x)

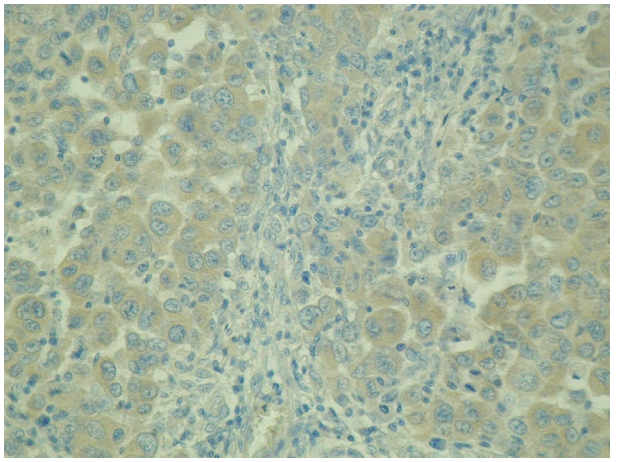

Figure 3. Positive staining: nm23 (400x)

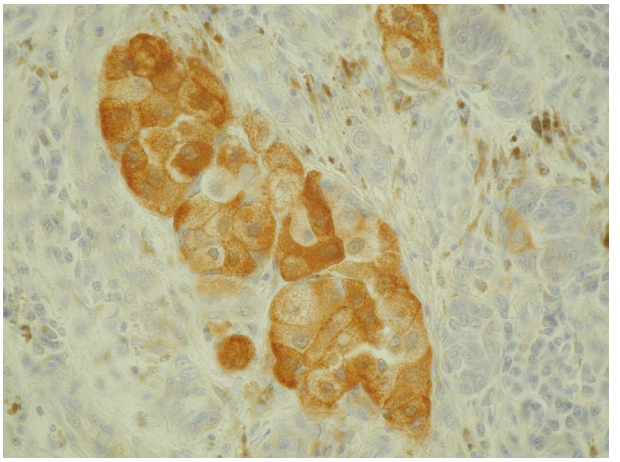

Figure 4. Positive staining: MMP-2 (400x)

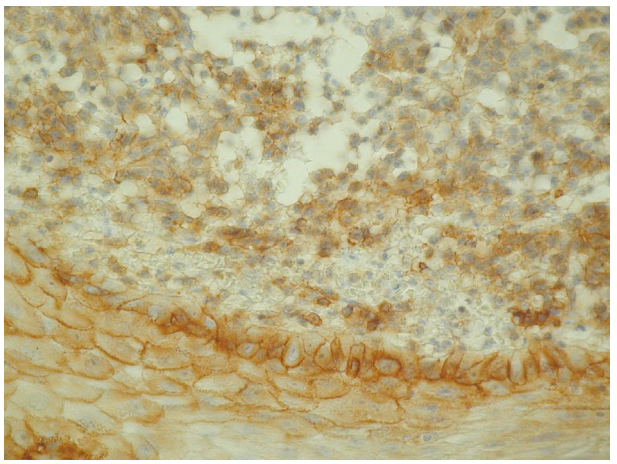

Figure 5. Positive staining: CD44 (400x)

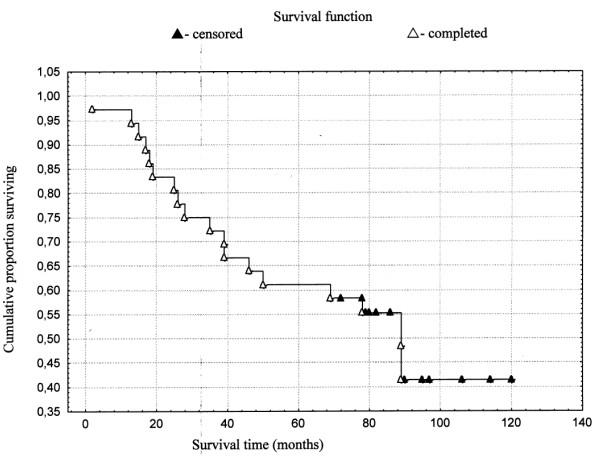

Figure 6. Graph of survival times vs. cumulative proportion surviving according to the Kaplan-Meier method.

melanin, positive reaction to PCNA, metalloproteinase 2 (MMP-2), CD44 glycoprotein and nm23 diphosphate nucleoside kinase.

The results are presented in Tables IV – VI.

Within the group of clinical and immunohistochemical factors the expression of nm23 diphosphate nucleoside kinase showed statistical correlation with survival time ($p=0.0202$).

Table IV. Statistical significance of clinical and immunohistochemical factors affecting survival time, assessed by Cox nonparametric proportional hazard regression model

Statistical survival analysis	Depended variable: survival – (months) Censoring observationes – 18 df = 8; p = 0.019 (p<0.05)			
Prognostic factor	n	Category	Relative risk (Exponent beta)	p
Age	36	continuous variable	1.0009	p=0.9641
Gender	30	female	1.56	p=0.5247
	9	male	0.64	p=0.5247
Limb location	22		2.61	p=0.7718
Lymphadenectomy	17		0.58	p=0.3222
PCNA	24	positive staining	2.26	p=0.6154
nm23	20	positive staining	0.08	p=0.0202
MMP-2	13	positive staining	0.81	p=0.4984
CD44	20	positive staining	0.44	p=0.3503

Table V. Statistical significance of histopathological and immunohistochemical factors affecting survival time, assessed by Cox nonparametric proportional hazard regression model

Statistical survival analysis	Depended variable: survival – (months) Censoring observationes – 18 df = 13; p = 0.0094 (p<0.05)			
Prognostic factor	n	Category	Relative risk (Exponent beta)	p
Lymph node metastases	15		3.77	p=0.1411
Breslow thickness	36	continuous variable	0.91	p=0.2366
Clark level	36		0.76	p=0.2856
SSMM type	34		0.47	p=0.4451
Ulceration	19		4.50	p=0.5666
Lymphocyte infiltration	23		1.38	p=0.7012
Regression symptoms	19		0.37	p=0.6919
Preexisting naevus	7		5.60	p=0.1218
Melanin pigment	34		1.28	p=0.7484
PCNA	24	positive staining	2.50	p=0.4702
nm23	20	positive staining	0.057	p=0.0452
MMP-2	13	positive staining	1.06	p=0.6191
CD44	20	positive staining	0.33	p=0.3833

Table VI. Statistical significance of histopathological factors affecting survival time, assessed by Cox nonparametric proportional hazard regression model

Statistical survival analysis	Depended variable: survival – (months) Censoring observationes – 18 df = 9; p = 0.024 (p<0.05)			
Prognostic factor	n	Category	Relative risk (Exponent beta)	p
Lymph node metastases	15		4.36	p=0.0175
Breslow thickness	36	continuous variable	3.51	p=0.0543
Clark level	36		0.37	p=0.1912
SSMM type	34		0.65	p=0.6598
Ulceration	19		1.09	p=0.9184
Lymphocyte infiltration	23		0.36	p=0.2267
Regression symptoms	19		0.96	p=0.9771
Preexisting naevus	7		1.90	p=0.4101
Melanin pigment	34		1.02	p=0.9788

The comparison of survival functions of a group of patients showing positive reaction to the existence of nm23 protein with a group of negative result according to the Kaplan – Meier method is presented in Fig. 7. No statistical correlation was found in the examined group between nm23 expression and the occurrence of distant metastases (Chi-square=2.78; p=0.95).

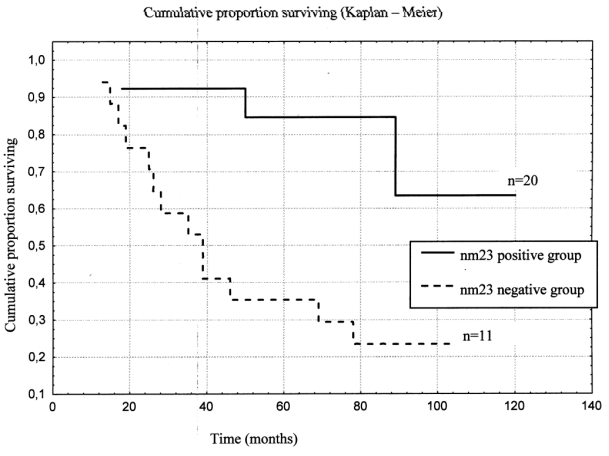

Figure 7. Graph of cumulative proportion surviving by nm23 group (Kaplan Meier – p=0.00076).

The remaining parameters such as: age, gender, lesion location, lymphadenectomy carried out along with tumour excision, positive reaction to PCNA, metalloproteinase 2 (MMP-2) and CD44 glycoprotein did not display statistically significant influence on the survival of examined patients. In the group of histopathological factors in comparison with immunohistochemical markers a positive result of the reaction to nm23 diphosphate nucleoside kinase presence (p=0.0452) displayed a statistically significant correlation with survival. Other factors such as: the thickness of lesion according to Breslow, the presence of lymphatic metastases at the beginning of treatment, the level of invasion according to Clark, histological type SSMM, the presence of ulceration, regression symptoms, preexisting naevus, melanin, positive reaction to PCNA, metalloproteinase 2 (MMP-2) and CD44 glycoprotein did not show any statistically significant influence on the patients survival.

Statistical analysis performed in order to assess the relationship between histopathological factors and survival time showed statistically significant influence on the existence of melanoma metastases in regional lymphatic nodes at the beginning of treatment for patients in whom lymphadenectomy was carried out along with tumor excision (p=0.0175).

Comments and discussion

The results show that 25% patients died within 28 months and 50% lived longer than 86.4 months. 61.5% patients survived 5-years or more. The ability to determine the group of patients with increased risk of disease progression is an important element in treatment planning.

The crucial prognostic factor in the examined group turned out to be a positive reaction result to nm23 diphosphate nucleoside kinase both in correlation with clinical and histological factors. Its importance is emphasized by many authors. Betke et al. performed an assessment of the presence of nm23 protein in benign naevi, malignant melanomas and melanoma metastases to lymphatic nodes and internal organs [31]. They found that in the group of patients they examined reduced nm23 expression is correlates significantly with survival time and with the presence of metastases (p=0.0003). Similar correlations of nm23 expression in melanoma have been confirmed by Florenes et al. [32], Lee et al. [30] and others [33]. Not all authors, e.g. van den Oord et al. [35], Easty et al. [36], Saitoh et al. [37] and Holmes et al. [38] have detected the correlation of nm23 expression in melanoma with survival time and metastases formation. Many authors performed the assessment of nm23 expression in other types of human cancer. Reduced levels of nm23 protein and correlation with survival time were detected in ductal breast cancer with regional lymphatic metastases [39]. In colon cancer there was a correlation between a reduced level of of nm23 and the occurrence of metastases to liver, however no dependence on the condition of regional lymphatic nodes was shown [40].

In the group of patients we examined the detection of nm23 expression in melanoma cells had some influence on prolonging survival time. The positive influence of nm23 protein expression on survival time in the examined group is consistent with literature data [30-34].

Van den Oord et al. [35] emphasize that demonstration of nm23 by routine immunohistochemical methods should not be the basis of the assessment of survival prognosis and postulate that only exact quantitative immunohistochemical methods can be the basis of such estimates.

During the course of the study it became impossible to assess nm23 for 5 patients (13.9%). The assessment of the remaining patients involved a comparison with hematoxyline/eosin – stained specimens. Only positive and negative reactions were assessed.

The immunohistochemical analysis confirmed the diagnostic value of HMB45 marker, however it did not have any prognostic value because as it presents an almost 100% specificity for melanoma.

The analysis of histopathological factors in the examined group of patients has shown an unfavourable influence of lymphatic metastases on survival time at the beginning of treatment. The research on the significance of lymphatic node status in melanoma has, in the recent years, led to changes in surgical treatment by introducing the SLN (Sentinel Lymph Node) biopsy. This method provides the possibility of verifing lymphatic node condition as a significant prognostic factor. It also allows to apply surgical treatment designed to spare lymphatic nodes and to detect early micrometastases within lym-

phnodes. SLN procedures have improved treatment results and the patients' quality of life [3-6].

The depth of invasion in mm according to Breslow and the level of invasion according to Clark, the most often emphasized prognostic factors, did not have any influence on survival in the analysed material. The significance of histological type of melanoma with surface type SSMM having better prognosis, preexisting naevus, ulceration and lymphatic infiltration was not demonstrated, either.

Within the group of clinical parameters (of which age, male gender, melanoma anatomical location on the trunk, shoulder girdle, neck or head are most often considered as unfavourable) none of them displayed any significant influence on survival time in the examined group. The influence of PCNA, MMP-2 and CD44 on survival time was not determined.

Immunohistochemical methods are the basic tool in the diagnosis and differentiation of pigment changes. This research has provided evidence as to the prognostic value of nm23 diphosphate nucleoside kinase in skin melanoma.

Conclusions

1. The expression of nm23 diphosphate nucleoside kinase has displayed statistically significant correlation with survival time in a group of patients with skin melanoma.
2. Despite the fact that the correlation between nm23 diphosphate nucleoside kinase and survival time has been proven, no statistical correlation between nm23 expression and the occurrence of distant metastases was detected.
3. The prognostic significance of the lymphatic metastases at the beginning of treatment was demonstrated in the group of pathological factors.

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Paper received: 27 February 2002
Accepted: 15 October 2002