Evaluation of IL-2, IL-6, IL-8 and IL-10 in Malignant Melanoma Diagnostics

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Abstract. *Aim: The aim of the present study was to evaluate the usefulness of four interleukins (IL-2, IL-6, IL-8 and IL-10) for melanoma detection and correlate these interleukins with sentinel node metastasis positivity. Patients and Methods: A group of 236 persons was assessed: 175 patients with melanomas and 61 healthy persons. Melanoma patients were divided to four groups according to Breslow score. We determined IL-2, IL-6, IL-8 and IL-10 in each plasma sample. Interleukin plasma levels were assayed using a Human Cytokine Milliplex Map kit. Measurements were performed using the Bio-Plex MAGPIX Multiplex Reader. Plasma samples were collected prior to surgery or any other form of treatment. All melanoma diagnoses were histologically verified. Results: We compared interleukin plasma levels in the healthy group and plasma levels in each Breslow score stage. In the first Breslow score stage, IL-2 ($p<0.0001$), IL-6 ($p=0.0004$) and IL-10 ($p<0.0001$) were positive. In the second Breslow score, stage IL-2 ($p<0.0001$), IL-6 ($p<0.0001$), IL-8 ($p=0.0017$) and IL-10 ($p<0.0001$) were positive. By comparing the group of positive and negative sentinel node metastasis, we observed a statistically significant difference in two interleukins: The median of IL-2 levels in the negative group was 5.88 pg/ml compared to 32.57 pg/ml in the positive group ($p=0.0005$). The median of IL-6 levels in the negative group was 4.80 pg/ml compared to 32.02 pg/ml in the positive group ($p=0.0048$). Conclusion: Interleukins IL-2, IL-6 and IL-10 are promising biomarkers of early-stage melanoma. IL-2 and IL-6 appear to be prognostic biomarkers.*

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Melanoma is one of the most aggressive forms of cancer and potentially lethal if not detected in early stage and treated properly (1). Melanoma is a malignant tumor originating from melanin-producing cells, the melanocytes. Its incidence is increasing worldwide (2). Interleukins (ILs) belong to the broader group of cellular messenger molecules, the cytokines, which allow cells of the immune system to communicate with each other and generate a coordinated, specific response to a target antigen. In tumorigenesis, these cytokines directly stimulate immune effector and stromal cells at the tumor site and enhance tumor cell recognition by cytotoxic effector cells. These findings promoted interleukin research in the diagnostic field. (3).The aim of the present study was to evaluate the utility of four interleukins, interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-8 (IL-8) and interleukin-10 (IL-10), for early melanoma detection and their plasma level correlation with sentinel node metastasis positivity. We also assessed their potential as prognostic factors.

Patients and Methods

Group of patients. This study was conducted between January 2011 and June 2014. We assessed a group of 236 persons: 175 patients (base group) with melanomas and 61 healthy persons (control group). We determined IL-2, IL-6, IL-8 and IL-10 in each plasma sample. For more detailed evaluation, melanoma patients were divided into four groups according to Breslow score. Next, we assessed interleukin plasma levels in the group of melanoma patients in relation to sentinel node metastasis and in the group without metastasis. All melanoma diagnoses were histologically verified.

Plasma samples. Peripheral venous blood was collected using the VACUETTE blood collection system (Greiner Bio-one Company, Kremsmünster, Austria). Plasma was separated by a 10-minute centrifugation step at 1300 x*g* and immediately frozen to –80˚C. Samples were thawed only once, just prior to analyses. Plasma samples were collected at the time of diagnosis, prior to surgery or any other form of treatment.

Methods used. Interleukin plasma levels were assayed using Human Cytokine Milliplex Map kit (Millipore Corporation, Billerica, MA, USA). Measurements were performed using the Bio-Plex MAGPIX Multiplex Reader (Bio-Rad Laboratories, Hercules, CA, USA).

Statistical methods. The SAS 9.2 (Statistical Analysis Software release 9.2; SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses. A summary of statistical findings for age and plasma levels of interleukins is presented. The Wilcoxon test was used to compare distributions of values between the groups of patients in each Breslow score stage with the group of healthy persons. The Wilcoxon test was also used to of the groups with or without sentinel node metastasis.

Results

The patient's characteristics concerning the age of melanoma and control groups are shown in Table I. Table II shows the overview of statistically significant positive interleukins in each Breslow score stage, including the corresponding *p*-value. A detailed evaluation of interleukin plasma levels in Breslow score stages is shown in Table III. We compared interleukin plasma levels in the healthy proband group and plasma levels of assessed interleukins in each Breslow score stage. In the first Breslow score stage, the results were significantly higher for the following interleukins: IL-2 (median=32.00 pg/ml, $p<0.0001$) compared to 4.60 pg/ml in the group of healthy probands, IL-6 (median=30.50 pg/ml, $p=0.0004$) compared to 4.80 pg/ml and IL-10 (median=32.63 pg/ml, $p<0.0001$) compared to 3.80 pg/ml. In the second Breslow score stage, the results were statistically significantly higher for all assessed interleukins: IL-2 (median=31.88 pg/ml, $p<0.0001$), IL-6 (median=32.05 pg/ml, $p<0.0001$), IL-8 (median=17.89 pg/ml, $p=0.0017$) and IL-10 (median=30.58 pg/ml, $p<0.0001$) compared to the healthy group. All assessed interleukins were also statistically significantly increased in Breslow score stages three and four. By comparing the group of positive and negative sentinel node metastases we observed a statistically significant change in plasma levels of two interleukins: The median of IL-2 levels in the negative group was 5.88 pg/ml compared to 32.57 pg/ml in the positive group ($p=0.0005$). The median of IL-6 levels in the negative group was 4.80 pg/ml compared to 32.02 pg/ml in the positive group ($p=0.0048$).

Discussion

Determination of interleukins started in the late 1980s in connection with the using of interleukins in anticancer therapy (4). The rate of human interleukin release and changes in serum levels of interleukins during oncological treatment have been studied extensively since the 1990s (5). After the year 2000, with the advancing knowledge of cell signaling pathways, the study of interleukin levels reached a new level of importance (6). In the 1990s, research also focused on the role of interleukins in the development of cancer, including melanoma (7, 8).

Table I. *Age characteristics of patient and control groups*.

Status	Count (N)	Age (years)			
		Mean	Median	Minimum	Maximum
Melanoma	175	57.1	58.0	16.3	89
Healthy persons	61	56.0	53.7	17.0	84

During the last three decades many studies examined the changes of interleukins in patient groups with cutaneous melanoma in comparison to healthy persons. Clinical results have indicated that early stage melanoma treatment is the most successful. The aim of future research in the field of interleukins as biomarkers for cutaneous melanoma is to find more sensitive and more specific biomarkers for early stage melanoma diagnosis.

In our evaluation we separated the entire melanoma group into four further subgroups using the Breslow scores. The Breslow score is based on measurements of the thickness of the melanoma. As primary tumor thickness increases, there is a significant decrease in survival (9). Currently, there are several systems available for clinical assessment and melanoma diagnostics. However, tumor thickness is one of the most important parameters in melanoma assessment (10).

Another important parameter in the evaluating of biomarkers is age dependence. In our group of patients, we observed that the levels of interleukins were dependent on age. This fact has to be taken into consideration in the evaluation of results. The age of the control group had no statistical significance ($p=0.8829$) in comparison to the entire group of melanoma patients. When stratifying patients into sub-groups based on the Breslow score, the age distribution of the smaller groups cannot be equal. Therefore, we also tested the statistical significance of the age differences between each group that was formed according to the Breslow score and the group of healthy persons. When the age difference was statistically significant ($p<0.05$), age adjustment was performed (11).

IL-2 has been used in anticancer therapy for many years. This therapy is effective in patients with melanoma and other types of cancer (12, 13). Other finding shows that the expression of IL-2 in the tumor microenvironment decreases

Table II. *Positive interleukins and p-values for each Breslow score stage.*

Breslow score (mm)	Count (N)	IL positivity (*p*-value)
<1	54	IL-2 (0.0001), IL-6 (0.0004), IL-10 (0.0001)
1-2	54	IL-2 (0.0001), IL-6 (0.0001), IL-8 (0.0017), IL-10 (0.0001)
2-4	47	IL-2 (0.0001), IL-6 (0.0001), IL-8 (0.0003), IL-10 (0.0001)
>4	20	IL-2 (0.0077), IL-6 (0.0015), IL-8 (0.0001), IL-10 (0.0001)

Table III. *Interleukin plasma levels in Breslow score stages.*

Analyte (units)	Status Breslow score (mm)	Mean	Median	Minimum	Maximum	*p*-Value
IL-2	Healthy	9.26	4.60	3.00	36.00	-
(pg/ml)	<1	21.95	32.00	4.68	46.50	<0.0001
	1-2	24.68	31.88	3.56	50.56	<0.0001
	2-4	23.03	33.25	3.00	46.78	<0.0001
	>4	18.85	18.50	4.15	55.00	0.0077
IL-6	Healthy	11.04	4.80	3.00	95.00	-
(pg/ml)	<1	22.95	30.50	3.50	178.0	0.0004
	1-2	32.77	32.05	4.30	362.0	<0.0001
	2-4	61.20	36.85	5.88	455.0	<0.0001
	>4	35.29	28.50	5.00	179.0	0.0015
IL-8	Healthy	10.34	8.06	3.00	32.00	-
(pg/ml)	<1	19.09	11.01	3.00	129.0	0.0623
	1-2	19.54	17.89	4.05	166.0	0.0017
	2-4	48.86	59.05	4.00	362.0	0.0003
	>4	55.21	49.00	4.20	642.0	<0.0001
IL-10	Healthy	5.64	3.80	3.00	115.0	-
(pg/ml)	<1	22.73	32.63	3.20	322.0	<0.0001
	1-2	25.03	30.58	4.85	288.0	<0.0001
	2-4	26.59	34.10	4.25	326.0	<0.0001
	>4	27.64	36.50	5.32	478.0	<0.0001

Table IV. *Interleukin plasma levels and sentinel nodes.*

Analyte (units)	Status	Count (N)	Mean	Median	Minimum	Maximum	*p*-Value
IL-2	Negative	48	10.16	5.88	3.12	36.76	0.0005
(pg/ml)	Positive	15	27.89	32.57	3.34	50.56	
IL-6	Negative	48	12.64	4.80	3.04	95.07	0.0048
(pg/ml)	Positive	15	32.77	32.02	3.78	362.0	
IL-8	Negative	48	12.51	8.47	3.25	40.60	0.3158
(pg/ml)	Positive	15	17.73	13.91	3.62	129.0	
IL-10	Negative	48	10.99	11.32	3.04	103.6	0.0995
(pg/ml)	Positive	15	13.51	13.21	4.56	198.6	

tumor growth (14). Information on the connection between plasma IL-2 levels and cutaneous melanoma prior to treatment remains relatively limited. However, a correlation has been found between elevated IL-2 levels and progression of gastric cancer (15) and non-small cell lung cancer (16). We observed elevated levels of IL-2 in our group of melanoma patients. Levels of IL-2 were statistically significantly higher even from the first stage of the Breslow score. IL-2 was significantly higher in the group of patients with the sentinel nodes metastatic positivity compared to the group of patients without metastasis. Our observation of increased levels of IL-2 looks similar in cases of both gastric and lung cancer. It seems that the plasma levels of IL-2 do not precisely reflect the mechanism of the anti-tumor influence of IL-2 in tissues, but the level correlates with cancer severity.

Several reports have described an association of serum IL-6 levels and human malignancies (17, 18). The increased plasma levels of IL-6 were described as a negative prognostic factor in patients with cutaneous melanoma measured prior to treatment (19, 20). The literature references regarding metastasis also note that elevated levels in melanoma patients with metastasis indicate a worse prognosis (21, 22) and shorter survival (23). We observed significantly increased levels in melanoma patients starting at the first stage of Breslow score scale. We have also observed significantly higher levels of IL-6 in the group with the sentinel nodes metastatic positivity compared to the group without metastasis. These facts correspond with previous literature findings. High level of IL-6 seems to be a negative prognostic factor.

In several studies IL-8 levels correlated with tumor burden in preclinical models and in patients with several types of cancer including melanoma. Certain authors indicate IL-8 as a potentially useful prognostic marker and as a biomarker for the monitoring of changes after anticancer therapy (24, 25, 26). In our group of patients the plasma IL-8 levels rapidly increased in third and fourth Breslow score stages. In our study, we focused on early stages of cutaneous melanoma. From this point of view IL-8 did not meet our expectations. Neither did we observe any statistically significant difference between the group with the sentinel nodes metastatic positivity and those without metastatic positivity.

In available studies, the plasma levels of IL-10 were elevated in advanced melanoma stages (27, 28). IL-10 was also used as a prognostic marker of anticancer therapy in advanced melanoma stages (29, 30). In our study, the IL-10 levels were significantly elevated from the first Breslow score stage and the elevation continued throughout all four stages. However, we did not observe any difference in the group of patients with the sentinel nodes metastatic positivity compared to the group without metastasis.

Conclusion

Based on Breslow score stage of melanoma, we can conclude that plasma levels of IL-2, IL-6 and IL-10 were significantly elevated, which means that IL-2, IL-6 and IL-10 are good candidate biomarkers of early stage of cutaneous melanoma. Especially, plasma levels of IL-2 and IL-6 were significantly higher in the group with sentinel node metastasis in comparison to the negative group, thus making IL-2 and IL-6 promising prognostic biomarkers.

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