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Melanoma inhibitory activity in Brazilian patients with cutaneous melanoma[*]

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Abstract: BACKGROUND: Melanoma inhibitory activity is a protein secreted by melanoma cells and has been used as a tumor marker. Increased Melanoma inhibitory activity serum levels are related to metastatic disease or tumor recurrence. Currently there are no studies on Melanoma inhibitory activity and cutaneous melanoma involving Brazilian patients.

OBJECTIVE: To evaluate the performance and feasibility of measuring Melanoma inhibitory activity levels in Brazilian patients with cutaneous melanoma.

METHODS: Blood was obtained from ten patients with proved metastatic cutaneous melanoma (Group 1), 15 patients resected for cutaneous melanoma without metastasis (Group 2) and 5 healthy donors (Group 3). Melanoma inhibitory activity was measured using a commercially available ELISA kit.

RESULTS: There was a statistically significant difference of Melanoma inhibitory activity levels between patients with and without metastasis (p=0.002), and between patients with metastasis and healthy donors (p=0.002). There was no difference between patients without metastasis and healthy donors (p=0.443).

CONCLUSION: Melanoma inhibitory activity is a tumor marker for cutaneous melanoma and the Melanoma inhibitory activity-ELISA test can be easily performed. Patients with metastasis have increased Melanoma inhibitory activity serum levels when compared to patients without metastasis and healthy donors.

Keywords: Follow-up studies; Melanoma; Tumor markers, biological

INTRODUCTION

Cutaneous melanoma (CM) is the fifth and seventh most frequent cancer found in men and women in the United States, respectively. It is estimated that 76,690 individuals in the United States will be diagnosed with CM, and the mortality rate associated with this tumor has been on the rise. [1]

Patients who have undergone the resection of CM should be followed up not only for the possible detection of its recurrence and metastasis, but also for the development of a new tumor, which occurs in 3% to 6% of all patients.[2] A complete clinical exam, including physical and imaging exams of the regional lymph nodes and systemic organs, is essential for patients with advanced disease. However, there is a lack of agreement about the selection criteria used for and the timing of the laboratorial exams and imaging studies when following patients with resected melanoma.[3] Depending on the institution or oncology group, different guidelines have been applied.[4] For example, the National Comprehensive Cancer Network (NCCN), the European Society for Medical Oncology (ESMO), the American Academy of Dermatology (AAD), the British Association of Dermatologists (BAD), and the Guideline for Management of Melanoma in Australia and New Zealand (GMMANZ) (1F) do not have specific recommendations regarding the use of

laboratory tests for the follow up of CM patients. On the other hand, the Swiss Melanoma Guideline (SMG), as well as the German Cancer Society and German Dermatologic Society, recommend that S100 blood levels be obtained in patients with clinical stage III–IV and III melanoma, respectively.[5-11] Nonetheless, serum markers such as lactate dehydrogenase (LDH), S100b, and melanoma inhibitory activity (MIA) have been used to monitor therapy response and to detect early recurrent and/or metastatic disease.[12]

When monitoring patients, financial considerations are important, especially when imaging exams are used. These examinations can add substantial costs to the management of the patient. In fact, the treatment of metastatic/advanced melanoma is one of the most costly treatments among all types of cancer.[13] Therefore, more effort should be made to develop successful approaches for the early diagnosis and treatment of CM, as well as for the early detection of metastasis.

MIA is an 11 kDa protein expressed and secreted by melanoma cells, but not melanocytes, as it is related to CM development and progression.[14,15] Increased MIA serum levels have been considered to be a reliable tumor marker in detecting and monitoring metastatic disease and for monitoring responses to therapy.[14,16-18] However, this is not accepted by all authors, and at this moment, no studies have been conducted regarding the role of MIA in CM involving Brazilian patients.[19] The kit that is used to detect MIA levels is commercially available, and it is measured via an enzyme-linked immunosorbent assay (ELISA) system; therefore, the equipment and resources necessary to perform the test are relatively simple.[17]

MIA binds to cell surface proteins that mediate cellular attachment, and it also binds to extracellular matrix proteins such as fibronectin, laminin, and tenascin. Therefore, MIA is associated with tumor cell detachment and invasion.[18] Following the introduction of migratory stimuli, MIA binds to the cell adhesion receptors, integrin $\alpha_4\beta_1$ and integrin $\alpha_5\beta_1$, which enable tumor cells to invade the tissues and metastasize.[18,20]

The aim of this study is to evaluate the performance and feasibility of measuring MIA levels among CM patients in Brazil.

METHODS
Patients
Patients were divided into one of three groups: Group 1 was composed of 15 patients without CM metastasis; Group 2 was composed of 10 patients with CM metastasis; and Group 3 was composed of 5 healthy donors (defined as individuals who were not diagnosed with cancer). All CM patients were treated primarily by surgical excision. The clinical

data of the patients and healthy donors are described in tables 1 to 3. Patients from Group 1 (n=15) had clinical stage I/II CM (Table 1). Patients from Group 2 (n=10) had clinical stage III/IV CM, and 40% of them were receiving chemotherapy treatment (Table 2). No patients underwent metastatic resection or irradiation therapy. This is not a prospective study and we do not have the patient's follow-up data. Individuals without cancer (Group 3) were also assayed to determine biologically "normal" MIA plasma levels.

Group 1 was composed of 8 females and 7 males with a mean age of 60.8 years, standard deviation (sd) of 13.3 years and ages ranging from 28-80 years (Table 1). Group 2 (Table 2) was composed of 2 females and 8 males with a mean age of 52.7 years (sd, ±7.7 years), with ages ranging from 39-61 years. The group of healthy donors (Table 3) was composed of 3 females and 2 males with a mean age of 40.2 years (sd, ±16.9 years), with ages ranging from 24-66 years.

TABLE 1: Clinical data and MIA serum levels of patients from Group 1 (no metastasis)

Patient number	Age	Gender	MIA serum levels (ng/ml)
1	47	M	11.1
2	52	M	8.4
3	28	F	8.7
4	80	F	7.1
5	65	F	6.4
6	69	F	7.9
7	55	M	5.4
8	60	M	6.1
9	46	M	8.6
10	65	F	6.2
11	64	F	8.1
12	67	F	3.9
13	70	M	6.4
14	76	M	4.3
15	68	F	3.5

TABLE 2: Clinical data and MIA serum levels of patients from Group 2 (metastasis)

Patient number	Age	Gender	Chemoth*	MIA serum levels (ng/ml)
1	41	M	Y	12
2	52	M	N	>30
3	55	M	N	>30
4	60	M	N	>30
5	39	M	Y	17.6
6	48	M	N	18.8
7	55	M	N	>30
8	57	M	Y	5.8
9	59	F	Y	7.9
10	61	F	N	>30

*Chemoth: Chemotherapy

TABLE 3: Clinical data and MIA serum levels of patients from Group 3 (healthy donors)

Donor number	Age	Gender	MIA serum levels (ng/ml)	Skin type*
1	66	M	4.0	III
2	32	M	5.1	III
3	31	F	6.4	II
4	48	F	4.1	II
5	24	F	8.6	V

*The skin type was classified according to Fitzpatrick's scale.

Specimen handling and ELISA assays

The samples were obtained with patient's informed consent and according to a protocol approved by the Ethics Committee of the Federal University of Mato Grosso do Sul, Campo Grande, MS, Brazil (protocol No. 1038). All participants were informed about the study and signed an informed consent form

Ten milliliters of blood was obtained from each patient. Blood samples were obtained and clotted at room temperature. The serum was collected following centrifugation, and samples were frozen immediately at –20°C until analysis. A standard ELISA method was performed using a commercially available ELISA kit (Roche® MIA-ELISA; Boehringer, Mannheim, Germany) according to the manufacturer's instructions. The cost of each kit was approximately R$3000.00 (reais, local Brazilian currency, which is approximately equivalent to USD $1,300.00); each kit can be used to conduct up to 96 tests. All samples were tested in duplicate.

Statistical analysis

Statistical analysis was performed using the Kruskal-Wallis and *post hoc* Dunn tests to compare MIA levels of patients with and without metastatic disease, as well as healthy patients' samples. A significance level of 5% and a confidence interval (CI) of 95% were used. Data were collected and analyzed using Microsoft Excel® (Microsoft Corporation, Redmond, WA, USA) and SPSS 11.0 for Windows® (IBM Corporation, Armonk, NY, USA).

RESULTS

MIA serum levels in patients from Group 2 (with metastasis) were higher when compared to patients in Group 1 (without metastasis) and Group 3 (healthy donors). There was a significant difference in MIA serum levels between groups 1 and 2 (p=0.005), and between group 2 and control (p=0.007). However, there were no statistically significant differences between group 1 and control (p=1.000). MIA levels in

the different groups are graphically represented on figure 1. Descriptive statistics is shown in table 4.

Mean MIA level in Group 3 was 5.6 ng/ml (sd, ±1.9 ng/ml) (Table 4). Therefore, it could be inferred that MIA "biologically normal values" vary between 1.8 ng/ml and 9.4 ng/ml (mean+- 2sd).

Four out of 10 patients from Group 2 had been receiving chemotherapy at the time when the blood was drawn. The MIA levels found in these patients (n=4; median MIA level = 10 ng/ml) were lower than the MIA levels found in patients who did not receive chemotherapy at the time of the blood draw (n=6; median MIA level = 30 ng/ml). Five out of the 6 patients who had not been receiving chemotherapy (83.3%) presented with MIA levels >30 ng/ml. The maximum MIA level observed in patients receiving chemotherapy was 17.6 ng/ml (Tables 2 and 4).

All patients from Groups 1 and 2 had skin color type I or II (based on Fitzpatrick's scale). Group 3 had two donors with skin color type II, two with skin color type III, and one with skin color type V. Interestingly, the MIA levels in healthy donors ranged from 4.0–6.4 ng/ml, except for the donor with skin type V, who presented with a MIA level of 8.6 ng/ml (Table 3).

DISCUSSION

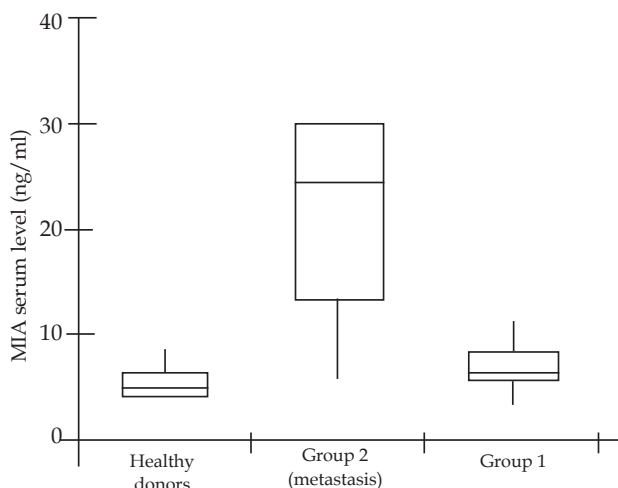To our knowledge, this is the first Brazilian study that has been conducted on MIA levels in CM patients.

FIGURE 1: MIA serum levels presented as median and quartile ranges. There was no statistically significant difference between the healthy donors group and the group without metastasis (p=1.000). However, differences between the group with metastasis and the other two groups were found to be statistically significant (group without metastasis p=0.005; healthy donors group p=0.007)

TABLE 4: Descriptive statistics of serum levels of MIA (ng/ml) for each group

Groups	Median	Mean ± Standard deviation	Smallest measurement	Maximum
No metastasis (n=15)	6.4	6.8 ± 2.1	3.5	11.1
Metastasis (n=10)	24.4	-	5.8	>30
Chemoth (n=4)	10.0	10.8 ± 5.2	5.8	17.6
No Chemoth (n=6)	30.0	-	18.8	>30
Healthy donors (n=5)	5.1	5.6 ± 1.9	4.0	8.6

*Chemoth: Chemotherapy

Tumoral markers are mainly used in the early detection of recurrent disease and/or metastasis when following up with patients.[21] In this study, we found statistically significant differences between MIA levels in patients with and without metastasis. However, there were no significant differences between MIA levels in patients with resected CM without metastasis and in healthy donors. Stahlecker *et al.* reported similar results.[17] They found that 5.6% of patients with stage I/II disease had increased MIA levels when compared to 60% and 89.5% of patients with stage III and stage IV disease, respectively. Interestingly, patients with advanced disease and low MIA levels were the ones who had had their blood drawn following metastatic surgery, irradiation, or chemotherapy. In our study, we also found that patients with metastatic disease who had received chemotherapy showed the lowest MIA levels. Mühlbauer *et al.* also demonstrated that patients treated with chemotherapy had lower MIA levels. The authors attributed this finding to the reduction of systemic malignant cells in these patients.[22] Therefore, it is not a surprise that many authors have considered the MIA test to be a highly sensitive and specific test, which is clinically valuable for the follow-up and treatment monitoring of CM patients.[23] MIA can detect melanoma recurrence before there is clinical evidence of the disease, even in patients receiving chemotherapy.[21,24] This is important because some studies demonstrated that the detection of disease recurrence at an early stage seems to be associated with a significantly better survival rate.[25] This means that the early detection of recurrent metastatic disease, and its subsequent surgical management, may be very important in the management of CM patients.

In a prospective study, Bosserhoff *et al.* analyzed MIA expression in 350 patients without metastatic disease.[23] Among 32 patients who presented with high MIA levels, 16 developed detectable metastasis or recurrence following the blood draw. However, none of the patients with low MIA levels developed metastasis or recurrence. This result demonstrates the high specificity of MIA in metastasis detection. In our study, only one patient (patient number 2) showed a high MIA level (>9 ng/ml) with no clinically detectable metastasis. Unfortunately, we cannot derive a conclusion based on this information, as we are not able to presently follow-up with the patient.

The early detection and management of recurrent malignant disease is important not only for the patient, but also for health care management. Treatment costs (TCs) associated with advanced CM are much higher when compared to the TC of an early-stage disease.[26] A Brazilian study assessed the TC of CM in the state of São Paulo, Brazil.[27] It was demonstrated that the initial stages of the disease (stages 0, I, and II) consumed 4.2% of the total budget resources, while advanced stages (stages III and IV) accounted for 95.8% of the total budget costs.

Borssehoff *et al.* demonstrated that MIA is strongly expressed and secreted by melanoma cells and, at lower levels, by some melanocytic nevi.[28] However, it is not secreted by normal melanocytes of the skin.[15] In this study, one of the healthy donors had skin type V (according to Fitzpatrick's scale) and presented with higher MIA levels (8.6 ng/ml) when compared to the other healthy donors with skin types II and III (MIA levels ranged from 4.0–6.4 ng/ml). To our knowledge, differences in MIA levels according to patients' skin type have not yet been discussed in the literature. Nevertheless, the presence of CM in patients other than Caucasians is uncommon.[29]

Other available tumoral markers for CM include S100 and LDH. Most studies on these markers have demonstrated that MIA has better sensitivity and prognostic potential than S100.[30,31] Moreover, most of these studies have measured S100 using immunoradiometric and immunoluminescent assays or polymerase chain reaction (PCR).[32-34] We know that the ELISA test is routinely used in most clinical laboratories throughout Brazil, as its application is very broad and ranges from oncology to mycology.[35,36] However, PCR and the other aforementioned assays are more specialized and may not be present in all laboratories, while ELISA is.

Cost is a very important issue when introducing a new marker or exam, especially in developing countries where resources are limited. One of the objectives of this study was to evaluate the feasibility

of performing MIA-ELISA in Brazil. The cost of each MIA-ELISA kit is approximately R$3000.00 (reais, local Brazilian currency, which is approximately equivalent to USD $1300.00). Each kit permits up to 96 tests to be performed at the same time. Therefore, the cost of each test is approximately R$30.00, plus the costs associated with the use of disposable materials such as syringes, needles and tubes. The kit is commercially sold by Roche® and was easily purchased by our team. The kit can be easily stored at 5°C to 10°C for up to 18 months, and the serum to be analyzed can be stored at –20°C for up to 1 month, according to the manufacturer's instructions. These features enable small laboratories to perform the test, as these laboratories would have fewer patients and samples to test. Compared to the prostate-specific antigen (PSA) exam that is currently used for the screening and monitoring of patients with prostate cancer, we can regard the MIA test as feasible for use among Brazilian CM patients.[37]

CONCLUSION

In conclusion, patients with clinically proven metastasis presented with MIA serum levels that were significantly higher when compared to those obtained among patients without metastasis and healthy donors. There were no significant differences between patients without metastasis and healthy donors in terms of MIA serum levels. Overall, this test is easy to perform and cost- effective.❏

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