„CAROL DAVILA" UNIVERSITYOF MEDICINE AND FARMACY, BUCHAREST
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MEDICINE

Histopathological and immunohistochemical study in malignant melanoma with tumor regression

PHD THESIS SUMMARY

Doctoral tutor:
PROF.UNIV.DR. STĂNICEANU FLORICA

Doctoral student:
CIOPLEA MIRELA DANIELA

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INTRODUCTION

Cutaneous melanoma (MC) is an aggressive skin tumor that can present a rare phenomenon in the absence of treatment: spontaneous regression, defined as the complete or partial disappearance of malignant cells. Although the mechanisms of regression in melanoma are not fully understood, current knowledge states that the host immune system has a key role in this process, characterized histologically by an intratumoral zone in which the malignant proliferation is partially or completely replaced by inflammatory cells, vascular hyperplasia and fibrosis. Regression is described in 10-35% of melanomas and the prognostic significance is difficult to assess. Some studies suggest that it is a sinister event, perhaps related to the enhancing effect that chronic inflammation has on tumorigenesis. However, this immune process is the basis of immunotherapy in melanoma with clear positive results.

CD8+ cytotoxic T lymphocytes are known to be primary effector cells in the host's antitumor response, but the interaction between the host's immune system and tumor cells in melanoma is complex and incompletely understood. Studies to date, which have elucidated the pathogenesis of tumor regression in melanoma, have led to the identification of new therapies and the development of key immune inhibitors for the management of advanced melanoma. New techniques for studying the tumor microenvironment may lead to a deepening of the immune study of malignant melanoma regression, which will develop new prognostic and predictive factors.

Over time, the studies carried out for tumor regression as a prognostic factor in melanoma have had conflicting results, until now many classifications of it have been reported, some opinions classifying it as a favorable prognostic factor, while others trust as a negative prognostic factor. Regression appears to have little influence on the biological behavior of melanoma, but it has some importance in grading the aggressiveness of melanoma.

CURRENT STATE OF KNOWLEDGE

1. Malignant melanoma: general data

Malignant melanoma is a malignant tumor proliferation, most commonly developed in the skin, originating in melanocytes, the cells containing melanic pigment. This tumor has a much higher incidence rate compared to other malignancies.

MM is the most aggressive skin malignancy, being the fifth most common tumor diagnosed in the United States, with a massive increase among the light-skinned population. The biggest problem with the increasing incidence of melanoma is that it is starting to be higher among the younger population compared to other malignancies.

Annually, over 300,000 people die from MM. An annual increase in the incidence of MM cases entails an increase in the mortality rate. The mortality rate for men has had a significant increase in recent years from 2.3/100,000 inhabitants in the 80s to 5/100,000 inhabitants in the 2000s, being much higher compared to that of women: from 2.2/100,000 inhabitants in the 80s to 2.9/100,000 inhabitants in the 2000s

The survival rate is the length of time a patient diagnosed and treated with a malignancy lives and is measured in a typical 5 or 10 year interval. In the case of MM, the survival rate depends on certain factors: location, stage, presence of metastases. Thus, in stages I-II the 5-year survival is 99.4%, for stage III 68% and for stage IV only 29.8%.

Stage	Breslow	5 years survival
	In situ	90-100%
I	< 1mm	80-90%
II	1-2mm	70-80%
III	2,1-4mm	60-70%
IV	>4mm	50%

Table 1 Survival at 5 years according to the Breslow index

A very important factor in the development of cutaneous malignant melanoma is represented by prolonged exposure to the sun. MM develops when the DNA in skin cells (melanocytes) is destroyed, most commonly under the action of ultraviolet radiation leading to genetic mutations or alterations, causing the mutant melanocytes to multiply rapidly, resulting in malignant melanoma.

BRAF mutations are more common in young patients with many melanocytic nevi and a history of sun exposure and those with superficial extensive melanoma (SSM). Mutations in KIT oncogenes are present in over 20% of cases of acral lentiginous melanoma (ALM) and mucosal ones. UV-induced mutations in the p53 suppressor gene are frequently observed in patients with stage IV MM, with a poor prognosis.

Annually, more than 7.8 million women and more than 1.9 million men use tanning devices, whose UVA and UVB radiations are much higher than those of the sun, which increases the risk of malignant melanoma. Most frequently, the use of sunbeds for tanning causes the appearance of melanoma, atypical nevi and solar elastosis on the lower limbs. Studies on the risks of exposure to UV radiation from tanning beds have led the European Commission and WHO's Scientific Committee on Health, Environment and Emerging Risks to state that there is no safe limit for exposure.

One of the risk factors for the development of malignant melanoma is the presence of melanocytic nevi, 25% of which develop on pre-existing nevi lesions. The risk of developing MM depends on the number, type and size of nevi. Some studies show that people with more than 100 nevus lesions have a 7 times higher risk of developing MM. Current guidelines recommend resection with 2 mm margins for ephelides if they present criteria such as asymmetry, irregular margins, color variations, diameter over 6 mm and if they have evolved rapidly (ABCDE criteria).

An important risk factor in the development of MM is family history. Studies have shown that families with inherited melanoma have an autosomal-dominant transmission pattern, with mutations frequently found in the CDKN2A or p16 gene.

Most commonly, MM is associated with obesity in patients with BMI over 30. Through melatobolic signaling, excess adipose tissue appears to induce hyperactivity at the level of the BRAFV600E oncogene

3. Precursor lesions

In general, the carcinogenesis of a malignancy involves intermediate lesions, which are found between malignant and benign. These lesions do not necessarily progress to malignancy, but present a risk of malignant transformation

Lesions that could lead to the development of malignant melanoma are:

- melanocytic nevi (donated nevi, atypical and dysplastic nevi): malignant melanoma can develop on any of the melanocytic nevi, but it usually occurs on healthy skin. Most frequently, MM develops on common melanocytic nevi (56.7%), and on dysplastic nevi 43.3%. An individual with a large number of melanocytic nevi has an increased risk of developing MM.

- congenital melanocytic nevi: the risk of developing MM on NMC is between 0.1 and 5%. The risk depends on the size of the congenital nevus, so very large congenital nevi have a higher risk of becoming malignant, between 2 and 5%. On these types of lesions, MM tends to develop early in the individual's life, with an average age of 15.5 years [42.

- solar lentigo and xeroderma pigmentosum: XP is an important risk factor for the development of MM, this being a rare disease with autosomal recessive transmission, in which the individual has a deficit in the level of repair nucleotides after destruction by UVA and UVB.

4. Clinical diagnostic criteria, classification, histopathology and immunohistochemistry of malignant melanoma

The most important diagnostic method in MM remains the histopathological diagnosis, which, in association with the clinical characteristics, is also the main classification method. MM is diagnosed incidentally or after the onset of symptoms, which include the appearance of a subcutaneous nodule, itching, bleeding or crusting of a pre-existing pigmented lesion. Until the onset of symptoms, which occurs after tumor progression and inflammation, MM is completely asymptomatic. The history of a patient suspected of MM should include questions about medical history (other skin malignancies), sun exposure, sunburn of the skin, booking in special salons, family history of MM.

The ABCDE rule represents the characteristics of integumentary tumor formation. Some studies show that this diagnostic method has a sensitivity of 91.6% and a specificity of 60.4%. These are:

- asymmetry
- B – the edges of the tumor (border)
- C - color
- D – diameter
- E – evolution (a more recent criterion that assesses tumor evolution)

The TNM classification is an important element in the diagnosis of MM both for the patient's prognosis and for its treatment and research. The 2018 World Health Organization (WHO) classification of MM classifies it as an independent biological entity with different clinical, histopathological and genomic characteristics. The new classification of MM

includes 9 distinct ways of its development, taking into account its clinical, histopathological appearance, epidemiology and genomics.

The new classification of melanocytic lesions is based on the influence of UV radiation, the origin of the tumor (the starting point of the tumor) and the genetic characteristics. To grade the cumulative damage caused by the sun from a histological point of view, the term solar elasto is used. In most countries, MM develops on the skin that is exposed to the sun, for a longer period or not, with a smaller number of MM that are not related to sun exposure.

Melanoame asociate cu expunerea la soareMelanomas associated with sun exposure Melanomas independent of sun exposure		Melanoame independente de expunerea la soare
Extensive surface melanoma/low melanoma -CSD		Melanoma Spitz
Lentigo maligna melanoma/high-CSD melanoma	acral	melanom
Desmoplastic melanoma		Mucosal melanoma
		Melanoma developed on a congenital nevus
		Melanoma developed on blue nevus
		Uveal melanoma
Nodular melanoma can occur through any of the above pathways		

Table 2. Classification of melanomas according to the mode of development

5. Prognostic factors in malignant melanoma

Currently, the prognosis of the patient with MM depends on the clinical stage correlated with the histopathological one. The clinical stage is established by a correlation between the histopathological aspect, the patient's clinic, imaging and laboratory analyses, which together establish the presence or absence of metastases. The correct correlation between the prognostic factors and the clinical stage is very important for the treatment and subsequent follow-up of the patient, as well as for the development of new strategies.

Clinical prognostic factors are represented by the age and gender of the patient, the skin type of the individual and the anatomical location of the tumor.

The most important histopathological prognostic factors are tumor thickness (Breslow), ulceration, the presence of microsatellite nodules and the number of mitoses. Other prognostic factors, less important, but which should be reported in the

histopathological report are: intra or peritumoral lymphocytic inflammatory infiltrate, lymphovascular and perineural invasion, regression and level of invasion (Clark level).

6. Tumor regression in malignant melanoma

Tumor regression is an immune process that leads to the total or partial disappearance of tumor proliferation. Like other tumors, MM can also present spontaneous tumor regression, a phenomenon that can create clinical and histopathological diagnostic problems, as cases have been reported in the specialized literature in which patients presented with metastases from MM without clear evidence of the tumor primary [89]. From the histopathological point of view, tumor regression can be absent, partial, segmental and complete. Tumor regression is a phenomenon due to the immune system that destroys tumor cells by means of lymphocytes. [90] Due to the association with intratumoral inflammatory infiltrate and the capacity for spontaneous tumor regression, MM is considered an immunogenic tumor.

The phenomenon of tumor regression in MM is a frequent one found in this type of tumor, with an incidence between 10-35% of all reported tumors. There is currently no consensus regarding the prognostic value of spontaneous tumor regression in melanoma, as some studies show that this is an unfavorable prognostic factor because some patients with MM present with metastatic disease and the absence of a primary tumor, other studies the presence of regression is not associated with positive sentinel lymph nodes. For this reason, at the moment, regression cannot be classified as a favorable or unfavorable prognostic factor.

PERSONAL CONTRIBUTIONS

7. Working hypothesis and general objectives

Although the data from the specialized literature give major significance with a positive prognosis to the immune response in malignancies of other organs, in fully regressed MM the prognostic importance of the immune response is controversial, most authors considering that it has an unfavorable prognosis, as a result of the presence of some cases reported in the literature of patients with lymphnodal and/or visceral metastases with complete regression of the skin tumor

This work aims to investigate from a histopathological and immunohistochemical point of view the presence of possible prognostic indicators, in order to correlate tumor regression with a prognosis in MM. Part of the research activity took the form of two scientific articles published in specialized journals indexed by ISI or PubMed.

The general objective of the doctoral thesis is to identify the morphological and immunohistochemical indicators with diagnostic and prognostic value in malignant melanoma with tumoral regression. To achieve the proposed objective, the research follows several stages:

- investigation of the immunophenotype of dendritic cells in the inflammatory infiltrate from tumor regression areas compared to that from non-regressed areas

- correlation of the presence and preponderance of dendritic cells in the inflammatory infiltrate in the regression areas of malignant melanoma and in the areas with inflammatory infiltrate in the non-regressed areas

- evaluation of the lymphocytic inflammatory infiltrate from tumor regression areas and non-regressed areas of the tumor

- the correlation between the presence of dendritic cells and the inflammatory infiltrate composed of T lymphocytes

- the presence of molecular adhesion makers, matrix metalloproteinase and metalloproteinase inhibitors and their role in tumor regression areas and non-regressed areas.

We expect that research data will demonstrate that tumor regression is a favorable prognostic factor in the evolution of malignant melanoma. The inflammatory infiltrate in regression areas has a particular composition compared to the intratumoral inflammatory infiltrate in MM without regression areas and depends on the expression of some signaling factors in the surrounding tumor melanocytes. Many of the studies performed to date are contradictory, with some suggesting that tumor regression in malignant melanoma has a good prognosis, others that regression is a poor prognostic factor.

8. General research methodology

In order to fulfill the previously mentioned objectives, we organized the present study in the form of a retrospective cohort study carried out on patients who underwent excision of skin fragments showing melanocytic lesions of the MM type in the sections of plastic surgery

and dermatovenerology of the Colentina Clinical Hospital, in -a side. -a span of seven years (2009 - 2016).

The examined material consisted of fragments of integument with tumor, taken, during the macroscopic orientation, from the skin pieces received in the Pathological Anatomy Service of the same hospital. They were routinely processed according to internal protocols and received complete histopathological diagnosis shortly after surgery.

The retrospective selection of cases was made by accessing the computerized database of the Department of Pathological Anatomy of the Colentina Clinical Hospital. The appendices from the accompanying sheet of the biological material taken from the patients were additionally checked, in which they express their consent including regarding the use for scientific purposes of surgically excised tissue fragments.

The group included in the study comprised a number of 60 patients selected consecutively, with the diagnosis of malignant melanoma with areas of tumor regression confirmed microscopically. From the pieces resulting from the case selection, they were divided into two groups: the group with areas from tumor regression areas and areas from the tumor from the same patient, thus resulting in 120 samples.

Fragments of interest (areas with tumor regression and tumor fragments without tumor regression) were extracted from the selected blocks and included in multi-tissue blocks. 3µ sections were made from the new multitissue paraffin blocks for usual stainings and 2µ for immunohistochemical ones, which were displayed on simple slides and dried for 2 hours at the thermostat. Subsequently, they were deparaffinized in xylene, rehydrated in ethanol solutions of increasing concentration and then in water and stained with hematoxylin-eosin stains according to the laboratory's recipe

All study cases received a complete immunohistochemical analysis using a panel of nineteen immunohistochemical markers. They included markers for the objectification of the inflammatory infiltrate composed of cytotoxic T lymphocytes (CD4, CD8, FOXP3).

Immunohistochemical markers such as CD1a, CD11c, Langerin were used for dendritic cells from the inflammatory infiltrate in regression areas, as well as from non-regressed tumor areas.

The presence of molecular adhesion makers (CEACAM1), inhibitors of matrix metalloproteinases and metalloproteinases and their role in areas of tumor regression and non-regressed areas (TIMP1; TIMP2, TIMP3, MMP1, MMP2, MMP3, MMP9) was evaluated by immunohistochemical tests.

To begin with, we took from the histopathological result of each patient the data related to his age and sex, the type of malignant melanoma, the Breslow index, the level of Clark invasion, the number of mitoses per mm^2, the presence or absence of ulceration and the location. All melanomas included in the study were extensive in surface.

The ages of the patients were grouped into 3 categories as follows: between 20-40 years; between 41-60 years and 61-90 years, also the number of mitoses: between 1-5 mitoses, 6-10 mitoses, over 11 mitoses. Absence of mitoses created a separate group.

Anatomical location of the tumor was grouped into 5 categories: head and neck, trunk, lower limbs, upper limbs, and no location (anatomical location could not be extracted from the accompanying note of the biopsy material).

Only cases with segmental and partial regression were included in the study, this being classified into regression areas over 75% of the tumor surface and tumor regression areas below 75% of the tumor surface.

The presence of T lymphocytes, CD4 and CD8, was scored as follows: score 0 – absent, score 1 – rare, score 2 – moderate number, score 3 – frequent score 4 – very frequent.

In the infiltrating lymphoid cells, the number of FOXP3-positive cells was assessed in both regressed and non-regressed areas as follows: rare (<20%), common (between 20% and 80%) and very common (>20%)).

9. Results

Demographic data about the studied group:

The group of studied patients includes 32 women and 28 men, the ratio between the sexes being 1.14:1 in favor of women. These data do not agree with the data from the literature, which report a higher prevalence among men, in this study the statistical significance being null. Depending on gender, the incidence of MM is higher among men, being 3.8/100,000 inhabitants, in women being 3/100,000 inhabitants, with a risk of developing this malignancy of 0.42% in men, respectively 0.33% in women. It appears that up to the age of 50, women have a higher incidence of MM, but after the age of 60, the incidence increases among men, who have a lower survival rate than women, which depends on certain biological factors.

The data obtained correspond to the general prevalence of the disease, which is more common in patients over 41 years of age, which represents more than half of the patients included in the study.

The correlation between age and gender is one without statistical significance in this study, the average ages of diagnosis of MM being similar, so the average age in men diagnosed with MM in this study was 61.35 years, and in women of 60.90 years.

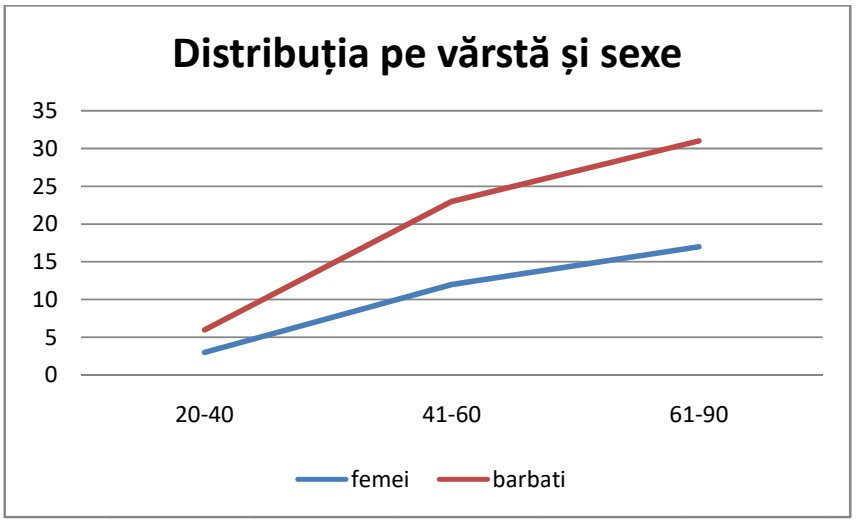


Fig.1 Correlation between age groups and sexes

The correlation between age and the Breslow index, the most important prognostic factor in MM, had no statistically significant value except for the 20-40 age group, where it had the lowest value (t-test, $p<0.032$) . As expected, the Breslow index was significantly higher in patients over 40 years of age compared to those aged 20-40 years.

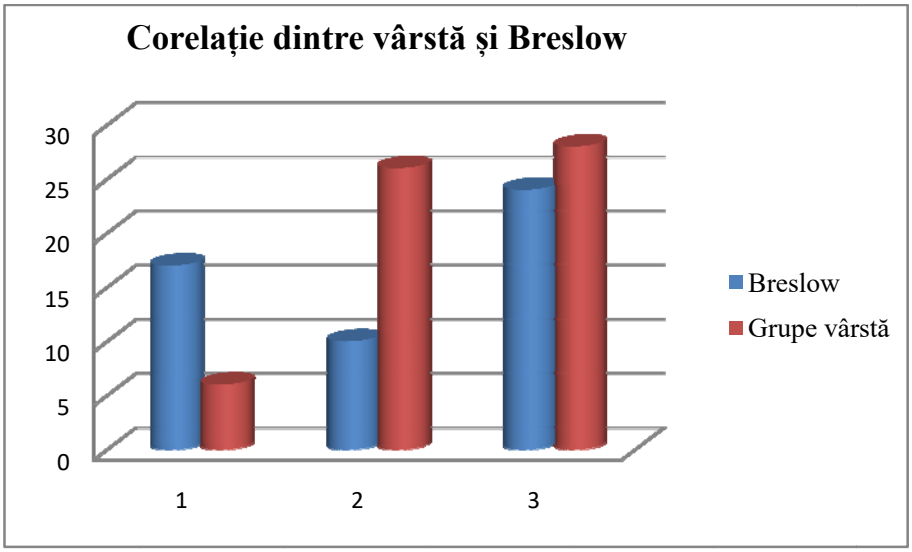


Fig. 2 Distribution of patients according to age and breslow

From the study carried out, it follows that older patients have a faster evolution of the disease, according to the Breslow index, which leads to the conclusion that the age of the patient with MM is an important prognostic factor in the evolution of MM.

In the study conducted, the correlation between genders and the Breslow index was statistically significant (t-test, $p<0.0006$)

Morphological and clinical data

The correlations between sex and location and age and location in the studied group are without significant statistical value, but a predominant location in the trunk was observed in both women and men.

MM ulceration is a negative prognostic factor. The five-year survival rate is reduced from 80% for non-ulcerated melanomas to 55% in the presence of ulceration for stage I melanoma patients and from 53 to 12% for stage II melanoma patients.

Melanomas with ulceration present appeared to have a higher Breslow index, with a mean tumor thickness of 3.47 mm, while thinner melanomas, with a mean tumor thickness of 1.23 mm, did not have ulceration.

In our group of regressed melanomas, the Breslow index ranged from 0.15 mm to 11.4 mm, the mean value being 2.57 mm. Of the total examined group, only 17 have a tumor thickness below 1 mm (thin melanomas), most of them having a thickness above 2 mm.

The current staging of MM is based on the AJCC (American Joint Committee on Cancer) staging system, implemented in January 2018 in the United States. Staging is based on the TNM classification (T-tumor, N-nodules, M- metastases). The tumor category is determined according to the thickness of the tumor - the Breslow index and the presence or absence of ulceration

Primary tumor [T]	Breslow [mm]
pTis	malignant melanoma in situ
pT1 ●pT1a ●pT1b	maximum tumor size 1mm●tumoră cu dimensiuni < 1mm fără ulcerație ● tumor with dimensions between 0.8-1 mm without ulceration or ● tumor size > 0.8 mm with ulceration
pT2 ●pT2a ●pT2b	tumor with dimensions between 1-2 mm ● tumor with dimensions between 1-2 mm without ulceration ● tumor with dimensions between 1-2 mm without ulceration
pT3 ●pT3a	tumor with dimensions between 2-4 mm ● tumor with dimensions between 2-4 mm without

●pT3b	ulceration ● tumor with dimensions between 2-4 mm without ulceration
pT4 ●pT4a ●pT4b	tumor size > 4 mm ● tumor with dimensions > 4 mm without ulceration ● tumor with dimensions > 4 mm without ulceration

Table 3. Tumor staging correlated with the Breslow index

The mitotic rate was between 1 and 20 mitoses/1 mm², with a mean mitotic index of 4.56 mitoses/mm2. As expected, there was a very strong correlation between mitotic index and Breslow (t test, tow tail P value 0.002).

Most studies of prognostic factors in MM have found that the Clark level of invasion has no prognostic significance after the Breslow index has been taken into account by multivariate analysis.

The Clark invasion level has 5 stages, detailed in the following table.

Clark	Nivel de invazie
Clark I	●also called melanoma in situ – melanoma cells are only in the epidermis
Clark II	●the presence of melanoma cells in the superficial dermis layer
Clark III	●the presence of malignant melanocytic cells in the deep dermis
Clark IV	●means that the melanoma has spread into the reticular dermis
Clark V	●means that the melanoma has invaded the hypodermis

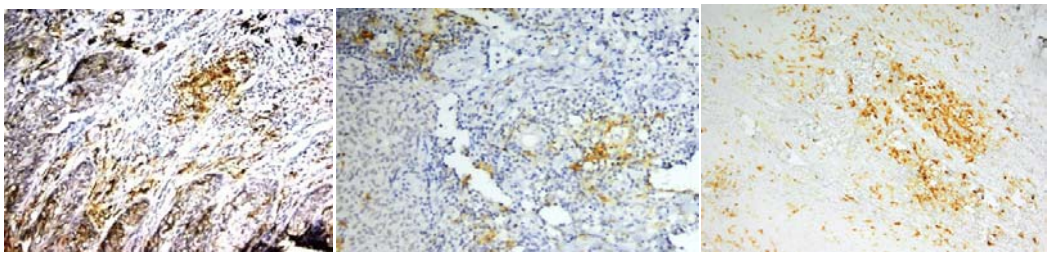
Table 4 Clark invasion level

Spontaneous tumor regression in malignant melanoma occurs in a small proportion of malignant melanomas, and it is important to understand the processes involved in its induction, as this may provide a guide to future therapies for this disease.

The distribution of the lot according to the presence of regression and the Clark index do not indicate significant differences. The distribution of the group according to the presence of regression and the mitotic index does not indicate significant differences (P 0.70), nor regarding the presence of ulceration.

Immunohistochemical markers in tumor regression areas correlations with nonregressed areas.

The difference between CD1a+ CD frequency in regressed and non-regressed areas is statistically significant (two-tailed P value=0.04).



A B C

Fig. 3 Distribution of dendritic cells: A. Nodular pattern; B. Arachnoid pattern. C. Diffuse pattern; CD1a 100X

In the studied group, the presence of positive dendritic cells for CD11c immunomarking did not have a statistically significant value in the regression areas compared to the non-regressed areas [two-tailed P value=0.5], but CD11c CDs were more numerous in the regression areas compared with the non-regressed ones.

Some studies have shown that CD4 T cells are associated with regression in primary melanoma and tumor rejection in adoptive transfer models. The mechanism by which it mediates its antitumor effects remains unclear, and some studies suggested that Fas ligand [FasL]/Fas interactions were involved.

As expected, in our study, CD4 positive cells were more numerous compared to CD8 positive lymphocytes. In non-regressed areas CD8 positive cells were more numerous compared to CD4 positive ones

In the infiltrating lymphoid cells, the number of FOXP3-positive cells was assessed in both regressed and non-regressed areas as follows: rare (<20%), common (between 20% and 80%) and very common (>20%) . The distribution of FOXP3-positive lymphocytes was also assessed in the regression areas and non-regression areas.

FOXP3 expression in tumor cells was statistically significant when correlated with the Breslow index [t test, two-tailed P value<0.038]. In thin tumors, FOXP3 was predominantly negative or had mild expression, whereas in thicker tumors, there was more intense expression of FOXP3.

In the studied group, it seems that the CD4 and CD8 positive cells are very different compared to their positivity for FOXP3, they have an inverse proportional relationship, in the areas where FOXP3 cells were numeroae, the dendritic cells had a reduced number and vice versa (t test, two -tailed P value<0.00021)

From the total number of cases, in the areas of tumor regression CEACAM1 was absent in 46 cases and weakly positive only in 14 cases. In the non-regressed areas, 17 cases were intensely positive, 14 moderately positive, 23 weakly positive and 6 negative. From this

it follows that there is a significant difference between the positivity of remaining cells in tumor regression areas compared to tumor cells in non-regressed areas

The expression of CEACAM1 in the regressed areas compared to the non-regressed ones does not present a statistically significant value, instead some studies have demonstrated that the expression for CEACAM1 has a statistically significant value in melanomas with regression compared to melanomas without tumor regression (t-test, $p<0.0001$)

There is a significant loss of CEACAM-1 expression in melanoma cells in regression areas, indicating that regression is not only the result of inflammation, but also of specific characteristics of some tumor cells that make them more sensitive to the toxic action of lymphatic cells.

In most cases, both in regressed and non-regressed areas, MMP1 showed weak to moderate positive expression. In tumor regression areas, MMP1 had immunohistochemical expression as follows: in most cases (46%) it was weakly positive, in 32% it was moderately positive, and in 22% it was intensely positive. In non-regressed areas 58% of them showed intense positivity for MMP1 in tu cells MMP-2 was diffusely positive in both regressed and non-regressed areas. MMP-2 was more intensely positive in non-regressed areas. In most cases, in areas of tumor regression, the expression of MMP-2 was moderate (53%), followed by cases with marked intensity (32%) and 15% were weakly positive. Comparatively, in the non-regressed areas, most cases had moderate intensity and the fewest were weakly positive, but there were no significant differences between tumor regression areas and non-regressed areas.

MMP-3 was diffusely positive in both the regressed and the non-regressed component, with no significant value between the two, although its expression seems slightly decreased compared to that in non-regressed melanomas.

MMP-11 in our study had slightly decreased expression, both in tumor regression areas and in non-regressed areas.

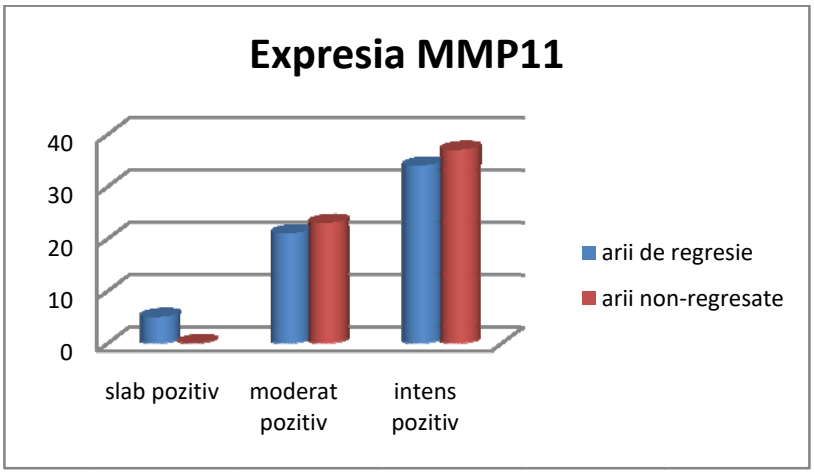


Fig.4 MMP11 expression in the studied group

In all cases, MMP had similar expression, both in regressed and non-regressed areas. MMP3 was the marker that showed slightly increased overexpression compared to the other metalloproteinases, followed by MMP-2 and MMP-11, MMP-13 and MMP-1 overexpression. Tumor stromal fibroblasts were also slightly more intensely positive in non-regressed areas than in regressed areas or showed similar expression in both components.

MMP2 was more intensely expressed in fibroblasts from non-regressed areas compared to those expressed in tumor regression areas (t-test, $p<0.00033$). MMP13 was more intensely expressed in the non-regressed areas compared to the regressed ones (t-test, $p<0.0006$). In the case of MMP1, MMP3 and MMP 11 there were no significant differences.

The expression of TIMPs had a greater variability in the non-regressed component compared to the regressed component of the studied group, both in tumor and stromal cells; there were cases of overexpression, similar expression, or decreased expression for each type of TIMP investigated. However, the majority of cases (60% for TIMP-1, 58% for TIMP-2, and 63% for TIMP-3) had TIMP overexpression in nonregressed versus regressed areas.

Conclusions

We performed a retrospective, cohort study, including 60 consecutive patients, diagnosed with malignant melanoma in the Pathological Anatomy Service of the Colentina Clinical Hospital, on clinical and histopathological bases. The study followed the immunohistochemical analysis of some cells from the inflammatory infiltrate in the regression areas tumor compared to non-regressed areas of malignant melanoma., based on microscopic evaluation.

The comparative analysis carried out allowed us to formulate a series of conclusions, of which we extracted the most important ones:

- MM is a malignancy that generally affects the elderly population, but can occur at any age, with a still high mortality rate
- In the studied group there is a discordance between the number of women and that of men, being included in the study 32 women and 28 men. These data do not correspond to those in the literature
- The age distribution respected the data from the literature, most of the patients in the study were over 60 years old
- In MM the tumor thickness (Breslow) is an important prognostic factor, which correlated with the patient's age, results that a smaller tumor thickness (thin MM) has a better evolution
- The location of the tumor has an important role in the evolution of MM, tumors located in the head and neck having a worse evolution.
- Characterizing CD and understanding its behavior will essentially contribute to the development of vaccines and management of skin diseases.
- More interesting is the association of the distribution pattern of dendritic cells in regression areas and the presence of dendritic cells within the non-regressed tumor mass.
- Langerhans cells have been consistently associated with favorable prognostic factors, thus constituting a promising parameter to be evaluated in the case of melanomas.
- CD4 positive cells were more numerous compared to CD8 positive lymphocytes.In non-regressed areas CD8 positive cells were more numerous compared to CD4 positive ones.
- FOXP3 is an interesting and promising target of molecular therapy because it appears to be an independent factor of aggressive behavior when expressed in tumor cells and is also deeply involved in the modulation of host immune defense.
- Tumor regression is correlated with an inhibition of FOXP3 regulatory T cells in the presence of increased numbers of dendritic cells. Data suggest that melanomas with high numbers of regulatory T cells and low numbers of dendritic cells are at higher risk for biologically aggressive behavior.

- An interesting observation is that the intensity of FOXP3 expression was higher in lesions that showed a diffuse pattern of positivity, while tumors that had only focal positivity had a lower intensity of FOXP3 expression
- CEACAM1 is a valuable marker in melanoma that can be used for a more complete description of tumor characteristics related to invasiveness and aggressive behavior. It is more intensely positive in thick melanomas and at the invasion front, indicating that CEACAM1-positive cells have a greater potential for invasion and metastasis.
- There is also a significant loss of CEACAM1 expression in melanoma cells in regression areas, indicating that regression is not only the result of inflammation but also of specific characteristics
- The expression of MMP3, MMP11, MMP13, TIMP1, TIMP2 and TIMP3 is decreased in the regressed areas compared to the non-regressed ones. These immunophenotypic characteristics indicate the existence of an intratumoral polymorphism, regression being the expression of intratumoral heterogeneity.
- On the other hand, although the results obtained did not have statistical significance for each individual marker, we identified a decrease in the expression of MMP1, MMP11, TIMP1, TIMP2 and TIMP3, for these cases there is a less aggressive biological behavior of melanomas with tumor regression .
- TIMP3 was overexpressed in all cases in the non-regressed areas compared to the regressed component.
- A trend of TIMP1 and TIMP2 overexpression was evident in non-regressed areas of melanoma compared to regressed areas.
- These findings support the hypothesis that the morphological differences identified in the spectrum of melanoma regression may correlate with prognosis, thus explaining the controversial findings in the literature regarding the biological and prognostic role of regression.
- MMPs and TIMPs are important molecules involved in tumor development, progression and metastasis with pro- and antitumor activity.
- Their correlation with regression in melanoma shows: (a) the regressed and non-regressed components are actually different tumor subclones and (b) in some cases of melanoma with regression (with a specific morphology), the biological aggressiveness of the tumor and implicitly the overall prognosis can be more favorable than that of melanoma without regression, thus offering the possibility of further stratification of these patients beyond AJCC staging.

- More studies are needed to establish comprehensive pathways as a gateway to identify new biomarkers for diagnostic or therapeutic purposes

Research data show that tumor regression is a favorable prognostic factor in the evolution of malignant melanoma. The inflammatory infiltrate in regression areas has a particular composition compared to the intratumoral inflammatory infiltrate in MM without regression areas and depends on the expression of some signaling factors in the neighboring tumor melanocytes. Many of the studies performed to date are contradictory, with some suggesting that tumor regression in malignant melanoma has a good prognosis, others that regression is a poor prognostic factor.

Our current data combined with previous studies on the diversity of regression in melanoma (both morphologic appearance, immune cell infiltration, and tumor cell immunophenotype), combined with divergent views on the prognostic significance of regression indicate that there is an identifiable category of regressed melanomas with a favorable prognosis and, in addition, prone to specific treatment.

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