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PHYSIOPATHOLOGICAL MECHANISMS IN CUTANEOUS MELANOCYTIC PROLIFERATIONS

PhD THESIS SUMMARY

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CUPRINS

INTRODUCTION 1
I. GENERAL PART 4
1 CUTANEOUS MELANOCYTIC PROLIFERATIONS - NEVUS, ATYPICAL NEVUS, MELANOMA - OVERVIEW 4
1.1. Melanocytes 4
1.2. Common nevus 5
1.2.1. Predisposing factors 5
1.2.2. Nevus classification 5
1.3. Atypical Melanocytic Nevus 8
1.4. Melanoma 10
2 MELANOMA 12
2.1 Epidemiology 12
2.2 Genetics 13
2.3 Histopathological Prognostic Factors of Melanoma and Tumor Staging 15
2.4 Molecules Involved in Melanoma Progression 20
2.4.1 E-cadherin, N-cadherin and epithelial mesenchimal transition in melanoma 20
2.4.2 VEGF 25
2.4.3 p53 26
2.4.4 CEACAM1 27
3 DERMOSCOPY OF CUTANEOUS MELANOCYTIC PROLIFERATIONS – NEVUS, MELANOMA 30
II. SPECIAL PART –PERSONAL CONTRIBUTIONS 33
4 WORK HYPOTHESIS AND GENERAL OBJECTIVES OF THE STUDY 33
4.1 Work Hypothesis 33
4.2 General Objectives of the Study 33
5 RESEARCH METHODOLOGY 34
5.1 Materials and Methods 34
6 DESCRIPTION OF THE STUDIED COHORT FROM A CLINICAL, DEMOGRAPHIC, AND HISTOPATHOLOGICAL PERSPECTIVE 46
6.1 Description of the Studied Cohort Based on Clinical Parameters 46

6.2 Description of the Studied Cohort Based on Clinical Parameters 52
6.3 Discussions 61
7 ANALYSIS OF THE STUDIED COHORT FROM A DERMOSCOPIC PERSPECTIVE 64
7.1 Characterization of the Studied Cohort Based on Dermatoscopic Description 64
7.2 Statistical Correlations between Dermatoscopic Criteria and Histopathological Parameters 82
7.3 Discussions 108
8 CONTRIBUTIONS REGARDING THE IMMUNOHISTOCHEMICAL CHARACTERIZATION OF THE STUDIED COHORT 111
8.1 Analysis of CEACAM1 Immunohistochemical Expression 111
8.2 Analysis of p53 Immunohistochemical Expression 117
8.3 Analysis of N-cadherin Immunohistochemical Expression 121
8.4 Analysis of E-cadherin Immunohistochemical Expression 129
8.5 Analysis of VEGF Immunohistochemical Expression 134
8.6 Discussions 147
9 CONCLUSIONS AND PERSONAL CONTRIBUTIONS 153
9.1 Conclusions 153
9.2 Personal contributions 156
Bibliography 157
APPENDICES 178

I. GENERAL PART - OVERVIEW OF THE CURRENT STATE OF KNOWLEDGE

Cutaneous melanocytic proliferations are a highly debated topic, with many discoveries over the years, yet still not fully understood. Continuous studies are essential, especially given the constant expansion of technology, providing us access to key information that can elucidate unknowns and clarify unclear aspects.

Melanoma represents a type of malignant melanocytic proliferation, being one of the most aggressive types of cancer. According to the 2019 statistics from the United States, melanoma is among the top 5 most common cancers, with its incidence continuously increasing. However, it ranks second in terms of survival rate compared to other cancers, likely due to its detection in early stages. (1)

Melanoma mortality has significantly decreased in recent years in the United States, with a 17.9% decrease between 2013 and 2016, after continuous increase until that point. This sudden change is believed to be primarily due to the introduction of new therapies for metastatic melanoma, as well as increased awareness and education resulting in early detection (71). Additionally, the improvement and widespread use of non-invasive diagnostic methods such as dermoscopy have contributed to this trend.

Mortality in early stages of melanoma is very low, with surgical cure being sufficient in most cases. However, there is a 2.4% proportion of stage I and II melanomas with a poor prognosis, and the reason for this subgroup of patients having an increased risk of tumor progression remains unknown (76). Personalized therapies targeting these high-risk patients would be ideal. Therefore, a detailed understanding of the pathophysiology of melanoma and the identification of the role of various molecules involved in its pathogenesis are necessary. New biomarkers are needed to stratify the risk of melanoma patients and facilitate early detection of at-risk individuals.

Immunohistochemical study of progression factors in melanoma is an area of ongoing research in recent years. With therapeutic discoveries such as immunotherapy, the study of molecules involved in the pathophysiology of melanoma is continually expanding, aiming to identify mechanisms that can lead to the detection of new therapies.

In addition to well-established molecules such as VEGF, E-cadherin, N-cadherin, and p53, this study also analyzes the cellular adhesion molecule CEACAM1. Recent studies suggest that

CEACAM1 plays an important role in tumor progression, increasing the risk of metastasis even in early stages of melanoma (2). Observing this aspect repeatedly, researchers in the field of melanoma are attempting to target and block this molecule through a monoclonal antibody directed against CEACAM1, theoretically aiming to halt tumor evolution (3). In this thesis, correlations have been made between the forementioned immunohistochemical markers and well-established histopathological progression factors used to evaluate and prognosticate the studied melanomas.

Dermoscopy is another current topic in the evaluation of melanocytic lesions that facilitates melanoma identification. Over the years, the foundations for diagnosing melanoma and differentiating it from benign lesions have been established, leading to the identification of tumors in early stages. Discrete changes in thin melanomas may not be visible clinically, but with the help of dermoscopy, they can be detected and monitored over time. Dermoscopic features of melanocytic lesions are continuously being discovered through intense studies in this field.

Efforts are being made to refine dermoscopic aspects for the identification of early signs and to identify at-risk patients. For this purpose, studies that simultaneously analyze dermoscopic and immunohistochemical aspects would be useful. One of the research directions of this thesis is represented by the correlations between dermoscopic aspects of melanoma and the immunohistochemical study of tumor progression factors (VEGF, p53, E-cadherin, N-cadherin, CEACAM1), which also represents a personal contribution to research.

Therefore, as future prospects, further studies comparing melanomas from a dermoscopic and immunohistochemical perspective would be beneficial for detecting patients at risk of tumor progression and, with the identification of innovative therapies, selecting treatments for specific patients without prior histopathological examination.

II. SPECIAL PART - PERSONAL CONTRIBUTIONS

4 WORKING HYPOTHESIS AND GENERAL OBJECTIVES OF THE STUDY

4.1 Working Hypothesis

The main purpose of this research is to conduct a detailed and comprehensive evaluation of the pathophysiological mechanisms involved in the tumor progression of primary cutaneous melanoma, as well as the identification of new prognostic and disease severity factors.

With an increasing incidence, melanoma presents a favorable prognosis only when detected in early stages, making early diagnostic methods for melanoma essential. In-depth knowledge of the pathophysiological mechanisms involved in the pathogenesis of melanoma can provide new information regarding tumor prognosis and the identification of patients at risk of disease progression. In this study, we evaluated melanomas and dysplastic nevi, along with common melanocytic nevi, from a clinical and demographic perspective, as well as using current histopathological, immunohistochemical, and dermoscopic methods. Through data analysis and correlations, we attempted to identify risk and prognostic factors for melanoma, alongside detecting patients with potential tumor progression.

4.2 General Objectives of the Study

In order to achieve this goal, the following general objectives were taken into account:

- Analyze the clinical and demographic aspects of cutaneous melanocytic lesions to identify the most relevant risk factors for melanoma.
- Describe the characteristic dermoscopic aspects of cutaneous melanoma and identify correlations between these aspects and histopathological parameters to facilitate early diagnosis and highlight disease severity indicators.
- Expand the knowledge domain regarding prognostic and progression factors of melanoma through the analysis of established histopathological parameters and current immunohistochemical markers.
- Identify correlations between the dermoscopic features of melanoma and immunohistochemical markers to detect imaging characteristics that may suggest tumor progression before histopathological evaluation of the excised cutaneous melanoma.

5 RESEARCH METHODOLOGY

5.1 Materials and Methods

This study represents a prospective observational study conducted between 2017 and 2019, with elements of a case-control design. It included 58 patients diagnosed with common melanocytic nevi, dysplastic nevi, and melanoma, who were evaluated clinically and dermoscopically at Colentina Clinical Hospital. After surgical excision, the lesions were processed and evaluated histopathologically and immunohistochemically at the Department of Pathology of Colentina Clinical Hospital and the Laboratory of Experimental Medicine and Fundamental Research in the Department II of Pathophysiology at the "Carol Davila" University of Medicine and Pharmacy in Bucharest.

The patient cohort was part of the CNCS-UEFISCDI research project, PN-III-P4-ID-PCE-2016-0641, crt no. 183/2017, within the PNCDI III program, a project funded by the Ministry of Research and Innovation, titled "Characterization of CEACAM1 Mechanisms in Melanoma based on Tumor Regression Study, an Innovative Approach for Optimizing Management of Thin Melanoma Patients" - CEACAMMEL. The study received approval from the Ethics Committee of Scientific Research at the "Carol Davila" University of Medicine and Pharmacy in Bucharest. (Appendix 2)

Inclusion criteria:

- Patients with clinical and dermoscopic suspicion of melanocytic lesion, later confirmed by histopathological examination - nevi, dysplastic nevi, melanoma
- Presence of complete histopathological description
- Completely excised lesions
- For the cohort with markers for immunohistochemistry - patients with tumors large enough to be evaluated immunohistochemically without jeopardizing the histopathological diagnosis

Exclusion criteria:

- Patients with clinical and/or dermoscopic suspicion of melanocytic lesions, disproved by histopathological examination
- Patients with lesions on mucous membranes for the dermoscopy cohort
- Patients without recorded dermoscopic images in the dermoscopy cohort

Selected cases of melanocytic lesions were clinically and dermoscopically evaluated at the Colentina Clinical Hospital. Patients were informed and initially signed an informed consent regarding the ongoing study. In the imaging evaluation, two types of dermoscopes were used, both with 10x magnification: Dermlite DL4 and Dermlite ProHR. Dermoscopic images were recorded and stored electronically for further detailed analysis and greater diagnostic accuracy.

Subsequently, cutaneous melanocytic lesions were excised and analyzed microscopically, with the specimens fixed in formalin and paraffin, a necessary step for conducting the histopathological examination. Among patients clinically diagnosed with cutaneous melanocytic lesions (nevi, dysplastic nevi, melanoma), only patients confirmed histopathologically with one of these diagnoses were selected for immunohistochemical analysis. Both the histopathological examination and immunohistochemical staining were performed at the Department of Pathology of Colentina Clinical Hospital. The interpretation and photography of immunohistochemical slides were later carried out at the Laboratory of Experimental Medicine and Fundamental Research in the Department II of Pathophysiology at the "Carol Davila" University of Medicine and Pharmacy in Bucharest. Some of the clinically evaluated patients could not undergo immunohistochemical evaluation, either due to insufficient tissue quantity (to avoid jeopardizing the diagnosis) or the absence of a paraffin block from histology lab. Multitissue blocks were made, which were subsequently sectioned and stained with immunohistochemical markers to highlight the melanoma progression: VEGF, p53, E-cadherin, N-cadherin, and CEACAM1.

As a result of these steps, a cohort of 58 cutaneous melanocytic lesions was obtained, including 35 melanomas, 6 dysplastic nevi, and 17 common nevi, which underwent dermoscopic analysis. Immunohistochemical staining was performed for 24 melanomas and 10 nevi (2 dysplastic nevi and 8 common nevi).

The working protocols for specimen orientation and processing for histopathological and immunohistochemical examination, as specified by the specialty of Pathology, were followed, as published in the Official Gazette no.723 of October 29, 2010 (221), issued by the Ministry of Health, and detailed in Appendix 3.

Clinical and Histopathological Evaluation Criteria:

In the study, the enrolled patients were analyzed based on several parameters.

Clinical and Demographic Indicators: age, sex, place of residence, tumor location, hemi-body, histopathological diagnosis.

Histopathological Indicators for the Melanoma Cohort: Breslow index, Clark index, mitotic rate, presence of ulceration, presence, quantity, and type of tumor infiltrating lymphocytes, presence of tumor regression, lymphovascular invasion, satellite tumor nodules, or perineural invasion.

Dermoscopic Evaluation Criteria:

Regarding dermoscopy, the 11 dermoscopic criteria described by Marghoob et al. (214) specific to melanoma were followed. These criteria were described for both the nevus and melanoma cohorts.

1. *Atypical pigment network*
2. *Angular lines*
3. *Streaks*
4. *Negative pigment network*
5. *Shiny white lines/Crystalline structures*
6. *Atypical dots and globules*
7. *Atypical blotch*
8. *Blue-white veil on elevated areas*
9. *Regression structures - scar-like areas, peppering (*granularity*), and blue-white veil on flat areas.*
10. *Atypical vascular structures* (dotted vessels, serpentine vessels, polymorphous vessels, red globules, milky-red areas)
11. *Peripheral tan structureless areas*

Immunohistochemical Evaluation Criteria:

- *VEGF*: Percentage of positive cells, measured in a hot spot, objective 40x; intensity of reaction (negative, weak, moderate, intense).

An index for VEGF was calculated by adding the intensity of the reaction and the distribution of VEGF staining. Intensity of staining was scored from 0 to 3 (negative, weak, moderate, intense), and the distribution of VEGF staining was scored from 0 to 3 (0%, 1-25%, 25-

50%, >50%). Negative VEGF index was represented by intervals 0-2, intermediate VEGF index by intervals 3-4, and strong VEGF index by intervals 5-6.

- *p53*: Considered present or absent based on the percentage of positive cells, measured in a hot spot, objective 40x, and intensity of reaction (negative, weak, moderate, intense).
- *E-cadherin, N-cadherin, CEACAM1*: Considered present or absent based on the percentage of positive cells, measured in a hot spot, objective 40x, and intensity of reaction (negative, weak, moderate, intense).

The statistical analysis and database were conducted using the IBM SPSS Statistics 21 program. The following statistical tests were used:

- ***Chi-Square Test:*** Used to evaluate the existence of a statistical relationship between two nominal/dichotomous variables.
- ***Phi Coefficient:*** Measures the statistical relationship between two dichotomous nominal variables (with two categories). It ranges from -1 to 1, expressing the direction and strength of the relationship between the two variables. Negative values indicate an inverse relationship, positive values indicate a direct relationship, and a value of 0 indicates no relationship.
- ***Cramer's V Coefficient:*** Measures the statistical relationship between two nominal or ordinal variables. Cramer's V coefficient ranges from 0 to 1, indicating the strength and direction of the relationship between the two variables. A value closer to 0 indicates a weak relationship, while a value closer to 1 indicates a strong relationship.
- ***Mann-Whitney U Test:*** A non-parametric test used to determine if there are differences between two independent groups when the dependent variable is either ordinal or continuous but not normally distributed.

6 DESCRIPTION OF THE STUDIED COHORT FROM A CLINICAL, DEMOGRAPHIC, AND HISTOPATHOLOGICAL PERSPECTIVE

Results and Discussions:

The 58 patients included in the prospective study were aged between 17 and 87 years, with a mean age of 55.21 years. The age group with the highest number of patients (24.14% - 14 patients) was the seventh decade (61-70 years). More than half of the patients were over 60 years old.

Regarding melanoma, the age group 61-70 years had the highest proportion of patients, accounting for 34% of malignant tumors (12 patients). The ages ranged from 33 to 87 years, with more than half of them being over 67 years old. The average age of patients with melanoma was 63.54 years, contrasting with the average age of patients with nevi, which was 42.52 years. Thus, it is observed that melanoma tends to be associated with older age compared to common nevi (statistically significant), which is a well-known risk factor for melanoma supported by existing literature and reconfirmed by the present study.

Regarding gender, the patient cohort was balanced, consisting of 29 females and 29 males. The melanoma cohort included 19 females and 16 males. It was observed that females presented more frequently with IB<1 mm, while males presented more frequently with IB>4 mm. Notably, male patients with melanoma exhibited more advanced stages of the disease, whereas females were diagnosed at earlier stages. This could be explained by the fact that females are more concerned about their physical appearance and tend to seek medical attention earlier compared to males, which is an advantage in their case.

Approximately 81% of the patients lived in urban areas, which could be justified by the higher access and easier reach to the medical system for these patients compared to those from rural areas.

The most frequent location of melanoma tumors was the posterior trunk, followed by the upper limbs, head, and neck, indicating areas more exposed to ultraviolet radiation. The most commonly affected hemibody was the right side, accounting for 60% of cases, with only 37.14% affecting the left side.

The studied cohort predominantly presented with incipient cases, with 42.86% having IB <1 mm (n=15), followed by 8.57% with IB = 1.01-2 mm, 20% with IB = 2.01-4 mm, and 28.57%

with IB >4 mm. The Breslow index is known to be the strongest indicator of tumor stage and progression.

Regarding the depth of melanoma infiltration, the most frequently encountered Clark index was IV, representing almost two-thirds of melanomas (65.71% of cases). Clark indices I, II, and III were found exclusively in patients with IB <1 mm, while Clark index IV was present in all IB categories. Clark index V was observed only in melanomas with IB >4 mm.

The included melanomas presented a depth of 0-5 mitoses/mm^2 in 60% of cases. It was noted that patients with up to one mitosis per mm^2 had the highest probability of being discovered at an incipient stage (all with IB <1 mm), whereas patients with over 5 mitoses/mm^2 presented a higher risk of advanced melanoma (all tumors in this category had IB >1 mm).

Ulceration, another risk factor described in the histopathological examination, was less frequent, present in less than one-third of cases in the studied cohort. Comparing patients with melanoma with and without ulceration in relation to the Breslow index, it was observed that ulceration was present in tumors with Breslow index over 2 mm. Tumors without ulceration had 60.87% of cases with Breslow index <1 (14 out of 23 without ulceration), indicating an incipient tumor stage. Therefore, melanomas with ulceration in the histopathological examination suggest a more advanced tumor form with an increased risk of progression and an unfavorable prognosis compared to melanomas without ulceration. Additionally, patients with melanoma had ulceration present only in cases with Clark index IV or V, indicating a deeper tumor depth.

Regarding the inflammatory infiltrate, 3 out of 4 melanomas in the studied cohort presented this characteristic in the histopathological examination. Among these, 42.31% had minimal infiltrate, and 50% had moderate infiltrate described. Comparing the type of inflammatory infiltrate, almost 70% had a non-brisk infiltrate, and only 30% had a brisk infiltrate. No statistically significant pattern could be established when comparing the inflammatory infiltrate with the Breslow index, but it can be observed that its presence increases with the thickness of the tumor in the studied cohort. As with contradictory results found in the literature, in our case, a slight tendency of this histopathological aspect to suggest an increased risk of tumor progression was observed.

Tumor regression was observed in approximately 35% of patients with melanoma, but without a tendency to associate with tumor thickness.

In the present study, 17.65% of melanomas exhibited lymphovascular invasion, 8.82% had satellite tumor nodules, and none of the melanomas presented perineural invasion. Both lymphovascular invasion and satellite tumor nodules were present in melanomas with tumor thickness over 1 mm.

7 ANALYSIS OF THE STUDIED COHORT FROM A DERMOSCOPIC PERSPECTIVE

Results and Discussions:

Statistical analysis of the studied cohort revealed certain dermoscopic characteristics that were specific to melanoma. Thus, the following dermoscopic features were more frequently encountered in melanoma cases and were statistically significant: crystalline structures, blue-white veil on elevated areas, peppering, atypical vessels in general, and specifically dotted vessels, serpentine vessels, milky-red areas, red globules, and polymorphous vessels. The other dermoscopic structures did not show statistical significance when comparing the cohort of melanomas with the control group (common nevi and dysplastic nevi).

In the specific analysis of melanomas based on established histopathological progression parameters, the following aspects were identified:

Atypical pigment network, as well as the streaks dermoscopic pattern, was significantly associated with a smaller Breslow index. This suggests that incipient melanomas more frequently exhibit an atypical pigment network and streaks compared to advanced melanomas.

The incidence of dotted vessels, serpentine vessels, and polymorphous vessels described in dermoscopy was observed to increase with the increase in the Breslow index, indicating an association with tumor progression and being more frequent in advanced melanomas.

Although not statistically proven, based on graphical representations, the presence of atypical dots, atypical globules, and streaks pattern in dermoscopy was associated with thinner melanomas.

Regarding the Clark index, atypical pigment network and regression structures were observed more frequently in melanomas located in shallower layers. Atypical vessels were found significantly more in deep melanomas with Clark index IV and V, although they were not exclusive (they can also be found in superficial melanomas).

It was noted that atypical vessels, polymorphous vessels, dotted vessels, serpentine vessels, and red globules were more frequently described in dermoscopy in cases with a higher mitotic rate. Therefore, these dermoscopic aspects suggest an increased risk of tumor progression.

Ulceration in the histopathological examination was inversely associated with the presence of an atypical pigment network, suggesting that the dermoscopic features described earlier

correlate with incipient, non-ulcerated forms of melanoma. In contrast, polymorphous vessels, dotted vessels, serpentine vessels, and red globules tend to be associated with the presence of ulceration, indicating a correlation of these dermoscopic characteristics with advanced, ulcerated tumor forms.

The presence of lymphovascular invasion in the histopathological examination correlated with the description of crystalline structures, dotted vessels, serpentine vessels, and polymorphous vessels in dermoscopy, suggesting their presence in a tumor stage with progression.

It was found that tumor regression described in the histopathological examination also correlated with peppering, which suggests tumor regression in the dermoscopic description. In addition, it was associated with the presence of the blue-white veil on elevated areas. However, without statistical significance but suggestive in graphical representations, other dermoscopic regression structures such as the blue-white veil on flat areas and scar-like areas were present in a higher proportion in lesions with regression described in the histopathological examination.

As mentioned in the article from the Romanian Journal of Clinical Research (225), the presence of atypical vessels in dermoscopy (polymorphous vessels, serpentine vessels, dotted vessels, etc.) stratifies patients with an increased risk of tumor progression and suggests the need for prompt and wider excision at the time of diagnosis due to the presence of more advanced forms of melanoma.

The present study brings us closer to the possibility of formulating algorithms that can predict melanoma stages through non-invasive imaging analysis called dermoscopy.

8 CONTRIBUTIONS REGARDING THE IMMUNOHISTOCHEMICAL CHARACTERIZATION OF THE STUDIED COHORT

Results and Discussions:

In the analysis of the immunohistochemical expression of the studied cohort, the expressions of known markers (VEGF, p53, N-cadherin, E-cadherin) were studied, as well as less-known markers such as CEACAM1 in the present cohort of melanomas, dysplastic nevi, and common nevi. The novelty of the research lies in the correlations between the expressions of these markers of tumor progression and the specific aspects of melanoma described in dermoscopy. Additionally, a novelty for research in Romania is the expression of the cellular adhesion molecule CEACAM1 in melanoma, and this work is one of the first studies to evaluate its presence in cutaneous melanocytic lesions.

From the analysis of CEACAM1 marker expression, it was observed that it is statistically associated with a high mitotic rate (over 5 mitoses/mm^2) in the studied cohort, as well as with the presence of ulceration in the histopathological examination. Moreover, there are statistical correlations with the presence of an inflammatory infiltrate in histopathology. These observations indicate that CEACAM1 expression suggests an advanced stage of melanoma, in which the tumor progresses rapidly, with an accelerated cell division rate, and an increased risk of metastasis, aspects also highlighted by previous studies.

Although without statistical significance, it is worth mentioning observations regarding tumor thickness and depth. It was found that CEACAM1 is more often positive among melanomas with a Breslow index over 4 mm, and its incidence increases with the increase of the Clark index.

The expression of CEACAM1 was observed to be more frequently elevated when dotted, serpentine, polymorphous vessels, or milky-red areas were described in dermoscopy. In contrast, atypical globules and scar-like areas were more frequently positive when CEACAM1 expression was negative. These remarks on dermoscopic aspects indicate the presence of CEACAM1 in advanced melanomas, in a state of tumor progression.

The p53 marker was statistically correlated with the presence of polymorphous vessels in dermoscopy, indicating a characteristic of advanced melanomas in progression. Positive p53 expression was more often found in cases with peppering, streaks, dotted vessels, atypical vessels, and milky-red areas.

Although without statistical association, it was observed that positive p53 expression occurred more frequently in cases with a high mitotic rate, as well as with the presence of lymphovascular invasion, satellite tumor nodules, and ulceration in the histopathological examination.

Another immunohistochemical marker of tumor progression analyzed was VEGF, whose expression was observed to increase with tumor thickness. In addition, the VEGF index correlated with the presence of a mitotic rate of over 5 mitoses/mm^2. Both observations support the idea that VEGF represents an index of advanced stages of melanoma and tumor progression, suggesting a negative prognosis.

Significant statistical associations were found between VEGF expression and crystalline structures, peppering, atypical vessels, dotted vessels, serpentine vessels, red globules, and polymorphous vessels described in dermoscopy. VEGF indicates neoangiogenesis, and the correlation with vascular dermoscopic features is logical, suggesting advanced tumor stages.

A statistical correlation was observed between VEGF expression and N-cadherin, both being markers of tumor progression, and without statistical association, but relevant from the perspective of graphical representations, negative CEACAM1 and p53 expression were more frequently associated with negative VEGF expression, while positive CEACAM1 and p53 were more often associated with positive VEGF expression (intermediate and intense).

In addition to previous observations, although without statistical significance, similar remarks were noted in the analysis of graphics regarding VEGF and its role as a tumor progression factor and indicator of advanced stages of the disease. Among these, the most relevant are the more frequent intense VEGF expression in cases with lymphovascular invasion and satellite tumor nodules described in the histopathological examination, suggesting the progression and metastatic risk of melanoma. In addition, VEGF is more intensely positive in melanomas with dermoscopic features such as streaks, or those without an atypical pigment network, atypical globules, or regression structures such as scar-like areas.

An intermediate VEGF index was more frequently observed in cases with regression, inflammatory infiltrate, and ulceration present in the histopathological examination, and regarding dermoscopic characteristics, it was observed more often in melanocytic lesions with milky-red areas, regression structures, atypical globules, scar-like areas, and those without streaks.

Negative VEGF expression appeared more frequently when lymphovascular invasion, satellite tumor nodules, ulceration, inflammatory infiltrate, or tumor regression were absent in the histopathological examination, and it was dermoscopically associated with the presence of an atypical pigment network, angular lines, and the absence of milky-red areas or streaks.

Regarding the other immunohistochemical markers, it was found that N-cadherin was more frequently identified in cases of melanomas. However, regarding E-cadherin, it had a slightly higher affinity for the nevi group, while in the melanoma group, it was more frequently present in melanomas with features of advanced tumor progression, both histopathologically and dermoscopically. Additionally, E-cadherin expression was positive more frequently in cases of lesions with positive CEACAM1 and N-cadherin expression, and with an increase in the VEGF index.

Atypical results regarding E-cadherin may be associated with the heterogeneity of the studied cohort and the limited number of patients. Additionally, the plasticity of tumor cells, which can modify their phenotype according to local needs, and can alternate between positive/negative states of the marker to meet their momentary needs and to invade deeper tissues during the proliferative stage, as noted by previous studies.

9 CONCLUSIONS AND PERSONAL CONTRIBUTIONS

9.1 Conclusions

Conclusions on Clinical and Pathological Risk Factors

From a clinical-pathological standpoint, the studied cohort highlighted the highest prevalence of melanomas in the 61-70 age group, located in sun exposed anatomical areas and the right hemibody, predominantly in urban dwellers. Aggressive forms of melanoma were more common in male patients.

Over 55% of melanomas in the studied cohort presented at least one histopathological criterion associated with a negative prognosis: Breslow index >1 mm, Clark index IV/V, presence of ulceration, and a mitotic rate over 5 mitoses/mm2.

Conclusions on Dermoscopic Aspects

Through the analysis of the melanocytic tumor cohort, statistically correlated dermoscopic features with melanoma were identified, such as crystalline structures, a blue-white veil on elevated areas, peppering, and atypical vessels (dotted vessels, serpentine vessels, milky-red areas, red globules, polymorphous vessels).

Regarding dermoscopic characteristics more frequently encountered in incipient melanomas, atypical pigment network, streaks, and dermoscopic regression structures were identified.

In contrast, advanced melanomas with a higher risk of tumor progression were more often associated with vascular dermoscopic features – dotted vessels, polymorphous vessels, serpentine vessels, red globules, and crystalline structures. These were correlated either with an increased Breslow index or with an increased mitotic rate, presence of ulceration, or lymphovascular invasion.

Peppering as a dermoscopic regression structure was significantly associated with tumor regression described in the histopathological examination. Additionally, microscopic regression was also correlated with the presence of a blue-white veil on elevated areas in dermoscopy.

Conclusions on Immunohistochemical Marker Expression

In the analyzed cohort, CEACAM1 positivity correlated with histopathological indicators associated with advanced melanoma stages, rapid progression, and relevant metastatic risk: increased mitotic rate, presence of ulceration, presence of inflammatory infiltrate, Breslow index over 4 mm, and increased Clark index.

In the analyzed cohort, CEACAM1 positivity correlated with dermoscopic indicators such as dotted vessels, serpentine vessels, polymorphous vessels, and milky-red areas; the absence of CEACAM1 correlated with atypical globules and scar-like areas, suggesting a possible association of CEACAM1 positivity with aggressive tumor progression.

Positive p53 expression was observed more frequently in advanced melanomas with a negative prognosis, associated with an increased mitotic rate, presence of lymphovascular invasion, satellite tumor nodules, and ulceration.

VEGF expression was associated with histopathological indicators of tumor progression and negative prognosis, such as Breslow index, mitotic rate over 5 mitoses/mm^2, and its intense positive expression was associated with the presence of lymphovascular invasion and satellite tumor nodules. Intermediate VEGF expression was more frequently found in melanomas with tumor regression, inflammatory infiltrate, and ulceration described in the histopathological examination, while negative VEGF expression was more frequent when lymphovascular invasion, satellite tumor nodules, ulceration, inflammatory infiltrate, or tumor regression were absent, suggesting its role as a marker identifying advanced melanoma cases.

Regarding dermoscopic features, VEGF expression was statistically correlated with the presence of crystalline structures, peppering, atypical vessels, dotted vessels, serpentine vessels, red globules, and polymorphous vessels. As VEGF indicates neoangiogenesis, the correlation with vascular dermoscopic indicators is expected, suggesting an advanced stage of melanoma. Intense VEGF expression was more frequent with the absence of atypical pigment network, atypical globules, or scar-like areas, and the presence of streaks. Negative VEGF expression was associated more often with atypical pigment network, angular lines, and the absence of milky-red areas or streaks. These observations are in line with the aggressive nature of melanoma suggested by positive VEGF expression.

N-cadherin expression correlated with histopathological indicators suggesting advanced tumor stages, such as Breslow index (most positive cases in the category over 4 mm), increased mitotic rate (over 5 mitoses/mm2), and the presence of a non-brisk inflammatory infiltrate. Increased N-cadherin frequency was found in patients with satellite tumor nodules and ulceration described in the histopathological examination. N-cadherin was observed to be associated with melanomas with regression, both dermoscopically (correlated with peppering) and histopathologically.

Correlations between N-cadherin expression and dermoscopic features were found with polymorphous vessels and milky-red areas, and more frequent with the presence of atypical vessels, serpentine vessels, dotted vessels, and red globules, all observations suggesting the presence of progressing melanoma forms.

E-cadherin expression was more frequent in both groups (nevi and melanomas), but with a slightly higher affinity for nevi. In contrast, in the melanoma group, E-cadherin was more frequently present in cases with advanced tumor characteristics both histopathologically and dermoscopically.

The study revealed significant associations between the evaluated IHC markers, highlighting that each marker can suggest tumor progression, aggressiveness, and an unfavorable prognosis through diverse mechanisms, leading to the heterogeneity of correlations with other indicators. The VEGF index was correlated with N-cadherin expression in IHC, and VEGF expression was more frequently negative when both CEACAM1 and p53 were negative. N-cadherin was more frequently present in tumors with positive CEACAM1 and p53 expression. Additionally, E-cadherin expression was more frequently positive in cases with positive CEACAM1 and N-cadherin expression and correlated with an increased VEGF index.

However, a limitation of the present study was the low number of patients for whom immunohistochemistry was performed, leading to differences in the presented characteristics, but not all had statistical significance. Further studies with larger patient cohorts and longer follow-up periods are necessary to complement the information presented in this research. Additionally, correlations with patient survival data would be useful to identify aspects related to melanoma mortality.

9.2 Personal Contributions

In terms of personal contributions, the most valuable element in terms of originality in this doctoral thesis is the immunohistochemical analysis of the CEACAM1 marker in melanocytic tumors, as its expression is considered promising and necessary for the staging and optimal management of melanomas.

In the doctoral study, correlations were made for the first time in the medical literature between the IHC expression of the CEACAM1 marker and the dermoscopic characteristics of melanocytic tumors, aiming for a comprehensive analysis of CEACAM1 expression in melanoma staging.

Within the cohort of melanocytic tumors, the correlation between the CEACAM1 marker's expression and histopathological and dermoscopic indicators, established for recognizing aggressive biological behavior in melanoma, was studied.

A novelty in the research on skin cancer, particularly melanoma, is the study of correlations between immunohistochemical markers of melanoma progression (p53, N-cadherin, E-cadherin, VEGF, CEACAM1) and described dermoscopic aspects. The utility of this comparison lies in discovering associations that can facilitate the detection of aggressive cancer forms or patients at risk of tumor progression, prior to lesion excision, through a non-invasive investigation such as dermoscopy. This can expedite the diagnostic and treatment process for patients at risk of rapid disease progression or advanced disease forms.

The author believes that such correlations between dermoscopy and the expression of various immunohistochemical markers can provide extremely valuable information in the future and expand the understanding of this continuously evolving type of cancer. It is likely that in the not-so-distant future, non-invasive imaging techniques like dermoscopy will replace invasive diagnostic and staging methods for melanoma, such as histopathological examination and immunohistochemistry.

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