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Development of New Markers in Squamous Cell Carcinoma of the Skin

Ph.D. Thesis Summary

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LIST OF PUBLISHED SCIENTIFIC PAPERS

1. ***Anamaria Grigore*** et al. Basosquamous carcinoma: an overview of actual protocol and the importance of basal and squamoid component. Romanian Journal of Military Medicine, Vol. 125, anul 2022, Nr. 3, pag. 391-400; doi: 10.55453/rjmm.2022.125.3.6 https://revistamedicinamilitara.ro/wp-content/uploads/2022/08/rmm_v3/ORIGINAL/Basosquamous%20carcinoma%20an%20overview%20of%20actual%20protocol%20and%20the%20importance%20of%20basal%20and%20squamoid%20components.pdf
2. ***Anamaria Grigore*** et al. Aesthetic principles in nasal reconstruction after squamous cell carcinoma resection. Romanian Journal of Military Medicine, Vol. 126, anul 2023, Nr. 3, pag. 312-316; doi: 10.55453/rjmm.2023.126.3.11 https://revistamedicinamilitara.ro/wp-content/uploads/2023/02/RJMM-vol-CXXVI-nr-3-din-2023-part-11.pdf
3. ***Anamaria Grigore*** et al. The immune response of cutaneous basosquamous- and squamous-cell carcinoma associated with sun exposure. Current Oncology, Vol. 31, anul 2024, Nr. 5, pag. 2481-2487, doi: 10.3390/curroncol31050185. PMID 38785466; PMCID: 11120243. IF 2.8 (2023) https://www.mdpi.com/2770154

INTRODUCTION

In recent decades, the incidence of cutaneous carcinomas, including squamous and basosquamous, has increased considerably [1]. This phenomenon is observed both in clinical practice and in specialized studies.

The present study aims to understand the molecular mechanisms underlying the onset and progression of these types of cancer. A better understanding of the etiopathogenesis of skin cancers can significantly improve the management of this pathology, reduce incidence and recurrence rates, decrease tumor aggressiveness, and thus develop new treatment lines that reduce hospitalization costs.[2].

Basosquamous carcinoma (BSC), also known as metatypical basal cell carcinoma, represents a distinct subtype of basal cell carcinoma (BCC) that exhibits a combination of clinical, dermatoscopic, and histological features of both cancer types: BCC and cutaneous squamous cell carcinoma (CSC), including a transition zone. BSC is considered to start as a BCC that undergoes genetic and epigenetic changes, leading to squamous differentiation through a transition from basal to squamous cells[3]. Known for its local invasiveness, high recurrence rate, and metastatic potential, BSC is considered one of the most aggressive forms of BCC.

The study hypothesizes a close link between skin aging, sun exposure, inflammation, immune response, and the occurrence of skin cancers[4]. Skin aging produces significant structural changes, including in its defense function. Sun exposure generates local inflammation that can stimulate the onset and progression of skin cancers while also generating a specific immune response.

To verify these hypotheses, the study used routine laboratory analyses collected from peripheral blood to evaluate immune response and inflammation. Nonspecific inflammation markers such as erythrocyte sedimentation rate (ESR) and elements from the complete blood count, such as the percentage of lymphocytes and the NLR (neutrophil to lymphocyte ratio), MLR (monocyte to lymphocyte ratio), and PLR (platelet to lymphocyte ratio), were analyzed to support these hypotheses.

NLR is a recently utilized inflammatory marker in diagnosing, prognosing, and assessing the severity of various diseases, including cancers[5]. Neutrophils, being essential immune cells, play a crucial role in inflammation, while lymphocytes are fundamental for immune function. NLR is considered an index for evaluating the systemic inflammatory response and has valuable implications in diagnosing and prognosing rheumatic diseases and cancers. Retrospective clinical studies have shown that peripheral blood NLR is a simple and accessible inflammatory marker capable of predicting the presence of rheumatic diseases and cancers[6].

MLR is another inflammatory marker investigated for its prognostic value in cancer. Monocytes are involved in inflammation and tissue remodeling processes associated with cancer progression. Studies have shown that increased MLR may indicate higher tumor aggressiveness and a poor prognosis in squamous and basosquamous cutaneous carcinomas[7].

PLR is used as a prognostic marker in cancer, with platelets playing an important role in coagulation, inflammation, and angiogenesis. An increased PLR may reflect a proinflammatory and pro-angiogenic tumor microenvironment favorable to tumor growth and metastasis. Studies suggest that an increased PLR is associated with a poor prognosis in various types of cancer, including cutaneous carcinomas[8].

Squamous cell carcinomas (CSC) and basosquamous carcinomas (BSC) are among the most common types of skin cancer. CSCs are more aggressive than BSCs and have a higher risk of metastasis. Inflammation plays a crucial role in the pathogenesis and progression of CSC. Inflammatory markers such as NLR, MLR, and PLR have been studied in SCC, and elevated values of these ratios are associated with more aggressive tumors and a poorer prognosis [9].

In the case of BSC, although usually less aggressive and rarely metastasizing, chronic inflammation and inflammatory markers can also influence the evolution of these tumors[10]. Limited studies have investigated NLR, MLR, and PLR ratios in BCC, but results suggest that these ratios may have prognostic value in BSC similar to CSC [11].

CURRENT STATE OF KNOWLEDGE

1. GENERAL NOTIONS ABOUT THE SKIN

The skin is a complex organ that represents an important barrier against the external environment, while also allowing communication with it. The skin as a whole should be viewed as more than an inert, impermeable layer against external aggressions, but rather as a complex, dynamic structure composed of cells, tissues, and matrix elements that mediate a series of vital roles.

Among these, the most important are the physical barrier function, protection against infectious agents, thermoregulation, sensation perception, protection against ultraviolet rays, tissue repair and regeneration, and creating the individual appearance of each person. These skin roles are achieved with the help of one or more main layers that compose it. Thus, the main layers of the skin are the epidermis, dermis, and hypodermis. These layers are actually interdependent on each other, each being connected to the surrounding structures to regulate normal cellular, molecular, and tissue function and structure [12]

The epidermis is the outer layer of the skin characterized by constant regeneration. It contains pilosebaceous units, nails, and sweat glands. The thickness of the epidermis varies between 0.4 and 1.5 mm, while the total skin thickness ranges between 1.5 and 4 mm. The epidermis is mainly composed of keratinocytes organized into four layers: basal, spiny, granular, and corneous. These cells differentiate from proliferative basal cells to fully differentiated keratinocytes, forming the keratinized corneous layer, the most superficial skin layer. Melanocytes are pigment cells derived from the neural crest responsible for melanin synthesis. They are found in the epidermis, hair follicles, iris, inner ear, and other structures. In the skin, melanocytes are located in the basal layer, forming the epidermal-melanin unit by associating with keratinocytes. These cells are essential for photoprotection and skin pigmentation [13].

The skin is not just an inert layer but a dynamic and complex structure composed of cells and tissues that perform a variety of essential functions for the body. The epidermis and melanocytes play a crucial role in skin protection, regeneration, and appearance, demonstrating the complexity and importance of this organ in maintaining the health and homeostasis of the human body [14].

The anatomy of the face is complex and varied, being composed of several layers of soft tissues, including the skin, subcutaneous adipose tissue, superficial fascia (superficial musculoaponeurotic system - SMAS), facial muscles, and deep tissue layers. These layers differ depending on the region, with the subcutaneous adipose tissue layer missing in the eyelids, lips, and nose. The superficial fascia includes the platysma muscle, superficial facial fascia, temporoparietal fascia, and epicranial aponeurosis, being responsible for facial expressions. The deep fascia covers the masticatory muscles, salivary glands, and main neurovascular structures [15].

The SMAS is a fascial tissue under the subcutaneous adipose tissue that connects the muscular fasciae to the dermis and transmits muscle contractions to the skin. The SMAS varies in thickness and structure in different regions of the face and neck. In the parotid gland and cheek, the SMAS is thick and continuous but thins and becomes difficult to distinguish in the cheek area. In the temporal region, the SMAS separates into fibers containing adipose tissue, while in the lower eyelid, there are anatomical variations in the SMAS structure [16].

The facial ligaments, such as the zygomatic and mandibular ligaments, anchor the facial skin and prevent the sagging of soft tissues. Several important ligaments include the temporal and supraorbital ligaments [17].

The facial nerve, a motor cranial nerve, exits through the stylomastoid foramen and branches within the parotid gland. The course and branches of the facial nerve are essential for the functioning of facial muscles and in surgical interventions, having particular importance in avoiding injuries during operations. The branches of the facial nerve include the frontal (temporal), zygomatic, buccal, marginal mandibular, and cervical branches, each with a specific course and function in innervating the facial muscles. There are eight danger zones in the head and neck area where motor or sensory nerves are more superficial and should be avoided during surgical interventions [17].

2. Etiopathogenesis of Cutaneous Cancers

Carcinogenesis is a complex, multi-step process involving the accumulation of genetic changes initially isolated in a single cell. These changes affect critical pathways in the regulation of cell division, senescence, apoptosis, and cellular interactions. Tumors are characterized by:

- Autonomy from growth signals through the activation of oncogenes.
- Avoidance of apoptosis by inactivating the p53 gene.
- Lack of response to proliferation-inhibiting signals.
- Unlimited replication potential due to telomerase activation.
- Angiogenesis induced by vascular endothelial growth factors.
- Metastasis through the inactivation of cell adhesion molecules [13]

Cell cycle regulation is carried out by cyclins, cyclin-dependent kinases (CDKs), and CDK inhibitors (CKIs). In carcinomas, frequent changes involve cyclins, CKIs, p53, and Rb, while CDK changes are rare [18]. UV radiation is a major risk factor for cutaneous carcinomas and malignant melanoma. The UV spectrum (100-400 nm) is divided into UVA, UVB, and UVC. UVA reaches the deep layers of the skin, generating reactive oxygen species and denaturing DNA. UVB is absorbed by the epidermis, inducing pyrimidine dimers and other DNA lesions. If these DNA damages are not repaired, they lead to mutations and cancer. Mutants of p53 are present in over 90% of squamous cell carcinomas and about 50% of basal cell carcinomas, being responsible for apoptosis resistance [6].

Squamous cell carcinoma is the second most common skin cancer after basal cell carcinoma. In the USA, about one million cases are diagnosed annually. The incidence of squamous cell carcinoma has increased, and the ratio between basal cell carcinoma and squamous cell carcinoma is now 1:1, in contrast to 3:1 in the past [19]. It predominates in men over 50 with fair skin and a history of UV exposure. The lifetime risk for white individuals is approximately 15%, influenced by increased UV exposure and longer life expectancy. Incidence increases in immunosuppressed patients who develop aggressive subtypes of squamous cell carcinoma.

Most squamous cell carcinomas develop from precursor lesions such as Bowen's disease or actinic keratosis [20].

A major risk factor directly correlating with the incidence of squamous cell carcinoma is UV exposure, more frequent near the equator and among veterans exposed to the sun during World War II. UVB exposure and long-term psoralen and UVA therapy significantly increase the risk. There is a correlation between ionizing radiation exposure and squamous cell carcinoma, especially in individuals prone to sunburn [21]. Aromatic hydrocarbons and arsenic are the most important. Smoking and alcohol consumption increase the risk of squamous cell carcinoma in the oral cavity. Chronic immunosuppression increases the incidence of squamous cell carcinoma, particularly in sun-exposed areas. Transplant patients have an 18-fold higher

risk. Incidence also rises in patients with leukemia or lymphoma. Squamous cell carcinoma is associated with post-burn scars, chronic ulcers, and chronic inflammatory pathologies leading to scarring [22].

Compared to squamous and basal cell carcinoma, basosquamous carcinoma (BSC) has relatively limited literature. Basosquamous carcinoma is considered an aggressive form of basal cell carcinoma with a higher risk of recurrence and metastasis. The first mentions in the literature date back to 1827 when Jacob first described rodent ulcer [23]. In 1894, a British pathologist from the Royal College of Surgeons described this type of tumor as having both basal cell and squamous cell carcinoma traits. In 1900 and 1903, Krompecher best defined basal cell tumors as "Basalzellenkrebse." In 1910, McCormack documented rodent ulcer cases, highlighting the mixed characteristics of the tumors. McCormack continued to publish similar cases in 1921. Between 1915 and 1924, Montgomery analyzed 119 consecutive cases of skin epitheliomas, discovering that 12.6% of these had "transitional" features [24].

In 2005, the World Health Organization (WHO) defined basosquamous carcinoma as a "tumor with infiltrative growth with areas of keratinization and/or formation of intercellular bridges within a typical proliferative stromal reaction." In the WHO classification, BSC is considered a basal cell carcinoma with squamous differentiation, while the National Comprehensive Cancer Network classifies it as a squamous carcinoma variant due to its prognosis and high risk of recurrence and metastasis.

Basosquamous carcinoma is common in elderly Caucasian men, especially in sunexposed areas. UV radiation is a major risk factor. In Germany, squamous cell carcinoma and its precursors are recognized as occupational diseases in cases of intense sun exposure, but basal cell carcinoma is not included in this category. Although epidemiological studies cannot establish a clear causal relationship, there is evidence that prolonged sun exposure may contribute to the development of BSC [25].

3. Hypothesis and General Objectives

Cutaneous carcinomas, especially basosquamous and squamous types, are a major medical concern due to their high prevalence and significant impact on patients' quality of life. This study focuses on both squamous cell skin cancer and basosquamous carcinoma, a specific type with distinct characteristics and important therapeutic implications.

The present study is based on a retrospective, observational, descriptive approach, aiming to identify and analyze molecular markers associated with basosquamous and squamous skin carcinomas. By analyzing patients diagnosed with basosquamous and squamous carcinoma in the Plastic Surgery Department of the Bucharest Emergency University Hospital from 2019 to 2021, this study aims to make significant contributions to understanding the pathology of these skin lesions.

The decision to include patients diagnosed with basosquamous carcinoma in this study stems from literature observations indicating a relatively small proportion of this carcinoma type compared to squamous carcinoma. This choice is based on the hypothesis that, despite the apparently lower prevalence, basosquamous carcinoma might have an increasing incidence, which could be highlighted through a detailed analysis of the included patients. However, basosquamous carcinoma is poorly defined, with a variable proportion of squamous cells, influencing its aggressiveness. In the future, basosquamous carcinoma could be more likely included in the category of squamous carcinomas and treated as a distinct entity.

The study was designed as a retrospective, observational, descriptive research conducted in the Plastic Surgery Department of the Bucharest Emergency University Hospital from 2019 to 2021. All consecutively hospitalized patients in the mentioned department with histopathologically confirmed cutaneous squamous or basosquamous carcinoma were included. The total number of patients included in the study was 132.

All collected data from patients with basosquamous and squamous carcinomas were centralized in a database created using Microsoft Excel; for statistical analysis, IBM SPSS Statistics 29.0.1.0 (IBM Corporation USA 2023) was used.

The t-student test for independent groups was used to compare data regardless of their distribution because the groups had more than 20 patients. A p-value $<.05$ was considered statistically significant. For categorical variables, Pearson's Chi-square test was applied to determine if there was a statistically significant difference ($p<.05$) between the expected and observed frequencies in the two groups.

All statistical data were reported according to the latest recommendations at the time of writing by the American Psychological Association (APA).

The study was conducted according to the principles of the Helsinki Declaration and was approved by the Ethics Committee of the Bucharest Emergency University Hospital. All patients provided informed consent before inclusion in the study.

Study 1: The Cumulative Role of Ultraviolet Radiation in the Initiation and Development of Carcinogenesis Through Skin Aggression, Aging, Immunosenescence, and Inflammation

The aging process is marked by the progression of physiological changes leading to the decline of biological, cellular, tissue, visceral, or entire organism functions. Ultimately, this process is associated with a susceptibility to diseases and death [26].

Chronic inflammatory processes associated with aging represent a key mechanism leading to "age-related" diseases. Subclinical inflammation at a low level leads to the overstimulation of the immune system, increasing mortality and morbidity among the elderly [27].

There are increasing studies and research showing a bidirectional relationship between the physiological aging process and changes in the immune system, a process known as immunosenescence [28]. This process is actually a consequence of aging and is represented by the gradual decline of immune cell functions and the body's ability to defend itself against pathogens. Immunosenescence, which leads to the body's inability to defend itself, further stimulates the immune system based on negative feedback. This results in the appearance of subclinical systemic inflammation [11].

Importantly for this study, the literature has demonstrated the importance of immunosenescence in several specific diseases, such as atherosclerotic disease, wound healing, tissue repair, and the body's ability to defend against stressors [29]. There are good exceptions, such as in the case of early tumors where oncogene activation stimulates cellular senescence and inhibits tumor progression. Certainly, this mechanism is eventually overcome, and the lack of a competent immune system leads precisely to the further development of the tumor [5].

In this study, we analyzed the impact that the patient's environment might have on the type of tumor they develop, and subsequently, we sought differences between these categories of patients regarding molecular markers.

Study 1 Results

In the study, the distribution of patients showed that 53% were male and 47% female. The majority of patients were observed in the age ranges of 70-79 years (37.9%) and 80-89 years (30.3%). Although the percentage is small, patients over 90 years were better represented than those under 60 years (9.1% vs. 8.4%). The majority of both male and female patients were aged between 70 and 79 years. However, there was a significant difference between the percentage of women over 90 years (6.8%) and men (2.3%). Regarding the environment, 52% of patients came from rural areas, and 48% from urban areas. A percentage of 80% of patients reported a high level of lifetime sun exposure, while only 20% had low exposure. The predominant diagnosis was BSC, found in 67% of patients, while 33% were diagnosed with CSC. In 80% of patients, the tumors were located in the head and neck area. An equal percentage of 8% of patients had carcinomas on the lower and upper extremities, and 4% had tumors in other locations.

Distribution by environment and gender revealed that 53% of men were from urban areas and 47% from rural areas. Among women, 42% were from urban areas and 58% from rural areas. Analyzing the location of the carcinoma and the patients' gender showed that 51% of those with carcinoma in the head and neck area were men and 49% women. In the lower extremities, 70% of patients were men and 30% women. Carcinomas of the upper extremities were equally distributed between genders, while 60% of carcinomas in other locations were in men and 40% in women. Regarding the type of carcinoma, 70% of men were diagnosed with CSC and 30% with BSC. Among women, 62.9% had CSC, and 37.1% had BSC. High sun exposure was reported by 77% of men and 84% of women, while low exposure was reported by 23% of men and 16% of women. Patients from urban areas had significant sun exposure in 73% of cases compared to 87% in rural areas, a statistically significant difference. Additionally, all patients with high sun exposure had carcinoma in the head and neck area. Among those with low exposure, 38% had tumors on the lower extremities and other locations, and 23% on the upper extremities, differences that were also statistically significant. Regarding the type of carcinoma and sun exposure, 67.9% of patients with high exposure had CSC, and 32.1% BSC. With low exposure, 38.5% of patients had BSC, but the differences were not statistically significant.

The ESR level was normal in 53% of men and 73% of women. An elevated ESR level was observed in 47% of men and 27% of women, with statistically significant differences. In BSC patients, 75% had a normal ESR level compared to 56% in CSC patients, where 44% had an

elevated ESR, a statistically significant difference. Urban patients had a normal ESR level in 56% of cases compared to 44% in rural areas. An elevated ESR was observed in 34% of urban patients and 66% of rural patients, a statistically significant difference. Regarding fibrinogen levels, 71% of men and 79% of women had a normal level. The mean ESR value was 23.58 mm/h in men and 27.58 mm/h in women, with no significant differences. The mean fibrinogen value was 371.20 mg/dL in men and 361.51 mg/dL in women, with no significant differences. The percentage of lymphocytes was significantly higher in women (25.38%) compared to men (21.89%). The mean number of lymphocytes was 1.67 in men and 1.95 in women, and the mean number of platelets was 229.91 in men and 261.04 in women, with statistically significant differences. The mean lymphocyte/monocyte ratio was higher in women (3.61) compared to men (2.82), with statistically significant differences. The mean glucose level was 116.23 mg/dL in men and 111.69 mg/dL in women, with no significant differences. The mean tumor diameter was 28.07 mm in men and 24.49 mm in women, with no significant differences.

Discussion

As observed in the results subchapter, the gender distribution in the cohort was almost equal. Most participants in the study were elderly, with 77.3% of patients being over 70 years old. These results are consistent with the literature. A Finnish study on 774 patients with cutaneous tumors showed that only 15.4% of patients were under 70, with most being between 70-79 years old (30.7%) and 80-89 years old (41.7%) [30]. This is important because studies have shown that the patients' age influences the average survival age from tumor diagnosis [31].

There were no significant differences between men and women regarding the age category, with both sexes having a similar frequency in the elderly categories. Additionally, an almost equal distribution was observed regarding the patients' environment. However, evaluating the cohort by sun exposure shows a clear predominance of patients with high exposure regardless of their environment (80%). Statistically significant differences by environment confirm the hypothesis that rural patients are much more exposed to the sun than urban patients. There were no significant differences between sun exposure in men and women.

Further analysis showed an approximately equal distribution of carcinoma types in the sun exposure groups, demonstrating that in this cohort, sun exposure did not influence the carcinoma type. The available literature did not find an article demonstrating a clear difference in the incidence of BSC and CSC based on sun exposure. It has been known for decades that

sun exposure causes acute skin changes such as burns, erythema, painful edema, and photodermatoses, as well as more serious chronic changes like actinic keratosis and cutaneous carcinomas: basal cell, basosquamous, squamous cell, and malignant melanoma. The population most affected by sun exposure includes rural workers, construction workers, and delivery workers [32]. A study by Hammont et al. showed that certain professions have a higher risk of developing skin cancer, such as construction workers, gardeners, and road and bridge workers. Although this study did not demonstrate a clear difference between sun exposure levels and carcinoma type, it did show a clear difference between the environments and sun exposure levels, indicating a clear influence of UV radiation in this cohort, especially in rural areas [34].

Initially, in the statistical analysis of cohort variables, we looked for differences in laboratory analyses between men and women. We found that female patients had a higher percentage of lymphocytes, an absolute number of lymphocytes and platelets, and a higher lymphocyte/monocyte ratio compared to male patients.

The statistical analysis revealed a higher ESR level in rural patients, correlating with higher sun exposure than urban patients. This was also correlated with a larger tumor size, a statistically significant difference, with an absolute value difference of 22.86±13.24 mm in urban areas vs. 29.50±21.96 mm in rural areas.

Furthermore, the statistical analysis showed a significant difference in lymphocyte percentage between patients with high and low sun exposure, with a higher percentage in those with high exposure. In contrast, the NLR ratio was higher in those with low sun exposure, further analyzed to identify which cells were higher. The statistical analysis revealed a higher number of neutrophils, indicating a lower lymphocyte percentage in those with low sun exposure.

Researching the available literature, we found studies evaluating tumor evolution through cancer sequencing. For example, one study demonstrated hundreds of evolving clones per square centimeter of skin, but an even higher frequency of mutated clones on sun-exposed skin [35]. Studies have also shown the role of inflammatory markers (elevated fibrinogen and ESR) not only in coagulation processes or acute systemic inflammation response but also in carcinogenesis. Our study found a higher frequency of elevated fibrinogen and ESR in patients with higher sun exposure, suggesting that these two laboratory markers could be used as prognostic markers in the future [36].

This theory of cancer development on an inflammatory background has been raised since 1828. In 1863, Virchow first described the presence of leukocytes in tumors and the connection between carcinogenesis and inflammation [37]. Subsequent studies showed that the systemic inflammatory response is involved in both the occurrence and development of carcinogenesis. Elevated fibrinogen and ESR levels have been extensively studied in other cancer types but not in skin cancers. This study lays the foundation for future research on these markers in skin cancers and their use as prognostic markers [38].

Conclusions

1. **Patient Profile**: Most studied patients are elderly, with 77.3% being over 70 years old. This aligns with a Finnish study on cutaneous tumors. Additionally, gender distribution was almost equal, and sun exposure was predominant among patients, with 80% having high exposure.
2. **Influence of Sun Exposure on Clinical Variables**: Statistical analysis highlighted a correlation between sun exposure and higher ESR in rural patients. Tumor size was also larger in rural compared to urban areas. Significant differences were found in lymphocyte percentages based on sun exposure, indicating the possible influence of UV radiation on the immune system.
3. **Prospective Inflammatory Markers in Skin Cancer Prognosis**: The study discusses using inflammatory markers like elevated fibrinogen and ESR as possible prognostic indicators in skin cancer. This opens the way for further research, suggesting a link between inflammation and carcinogenesis. Given the absence of such studies in skin cancers, this study proposes to contribute to future research and identify relevant prognostic markers.

Study 2: Comparative Analysis of Clinical and Paraclinical Data Based on Skin Carcinoma Type

BSC, a rare and aggressive non-melanoma skin cancer, shares characteristics between basal cell carcinoma and squamous cell carcinoma. The controversy around its classification, pathogenesis, histological morphology, biological behavior, prognosis, and management has generated interest in the medical community [39]. Literature highlights the importance of using dermatoscopy, deep incisional biopsies, and immunohistological techniques (such as Ber-EP4) in evaluating clinically suspect lesions. Early diagnosis using these methods contributes to a better prognosis for patients with basosquamous carcinoma. Surgical interventions, especially

wide excision and Mohs micrographic surgery, remain preferred treatment modalities for BSC. However, the need for in-depth research on vismodegib, a Hedgehog pathway inhibitor, through extensive controlled studies is emphasized. Vismodegib can offer an alternative solution for unresectable or difficult-to-treat BSC cases, especially those with locally advanced presentations. The incidence rate of BSC, situated between 17% and 27%, represents a relatively limited range but at the same time reflects a significant proportion within the population. Analyzing these figures, we can draw several conclusions:

1. BSC is considered a rare type of skin cancer, given that its incidence is below 3%. This places it in a less common category compared to other forms of cutaneous cancer [40].

2. The relatively narrow incidence range (17% - 27%) from the literature suggests a certain uniformity concerning the diagnosis of this particular cancer type. This consistency can facilitate the management and research of this cancer type, providing a relatively clear framework for doctors treating such cutaneous cancers [24].

3. Given the rarity of this cancer type, early diagnosis and careful investigations become crucial. Due to its hybrid characteristics between basal cell carcinoma and squamous cell carcinoma, basosquamous carcinoma may require a more specific approach for its evaluation and treatment.

4. Incidence data provide an important starting point for future research. Additional epidemiological and clinical studies may be needed to better understand the risk factors, clinical evolution, and optimal therapeutic options for basosquamous carcinoma.

Results

Regarding the mean age of patients, no significant differences were identified between the BSC group (74.86 ± 9.53 years) and the CSC group (76.14 ± 12.50 years).

Referring to biological markers, a higher mean ESR value was evident in CSC (27.54 ± 18.48 mm/h) compared to BSC (20.65 ± 16.92 mm/h), a statistically significant difference ($t(130) = 2.059$, $p = 0.041$). However, the mean levels of fibrinogen, neutrophil percentage, lymphocyte percentage, monocyte percentage, eosinophil percentage, basophil percentage, lymphocyte count, platelet count, PLR, NLR, and LMR showed no significant differences between the two carcinoma types.

The maximum tumor diameter was also examined based on carcinoma type. No statistically significant differences were identified concerning the maximum tumor diameter ($t(130) = -1.426$, $p = 0.156$) between the basosquamous and squamous cell carcinoma patient groups.

Study 3: Comparative Analysis of Surgical Closure Methods Based on Age, Tumor Size, and Tumor Type

Cumulative radiation exposure and aging processes are recognized factors for structural skin changes, contributing to the predisposition to cutaneous carcinomas. The choice of surgical technique must be personalized, adapted to the patient's age, tumor size, and location to achieve an aesthetic and functional result.

Post-aging skin structural modification contributes to its laxity, an important factor in choosing the method of closing the defect resulting from surgical excision.

Our results showed a predisposition of these carcinomas in individuals over 70 years old. More than half of the patients (52.4%) benefited from direct lesion closure. Local flaps were used in 37.1% of cases, and skin grafts were necessary for only 10.5% of patients. Skin grafts were mainly used for frontal, jugal, nasal, palpebral, and temporal locations. Almost half of the patients (47.7%) had large tumors (diameter > 20 mm), and medium-sized tumors (1120 mm) represented 34.6% of cases. Small tumors (0-10 mm) constituted only 17.7% of the studied cohort.

Small tumors were directly closed in most cases (78.95%), while direct closure was used in 51.25% of cases for medium-sized tumors. For large tumors, the percentage of direct closure significantly decreased.

Most patients presenting to the plastic surgery department had large tumors (>20 mm), highlighting the necessity for medical education for early diagnosis and treatment of cutaneous tumors, especially among the elderly who are more prone to developing cutaneous carcinomas.

Discussion

In our clinical practice, we observed a significantly increased incidence of BSC compared to the number of cases reported in the literature. This observation led us to conduct a detailed and comparative analysis between CSC and BSC, considering variables such as patient age, lesion size, sun exposure, and changes observed in peripheral blood analyses.

BSC represents a particularly interesting subject due to its complex and controversial characteristics. This type of cancer presents features of both basal cell carcinoma and squamous cell carcinoma, giving it a unique status in the oncological field. Its aggressive nature and rapid evolution resemble CSC in many ways, manifesting a high risk of recurrence and metastasis.

A notable peculiarity of this carcinoma type is that to date, oncological guidelines have not developed a specific treatment for BSC. The absence of a well-defined protocol can generate significant challenges in managing and adequately approaching this complex condition.

Besides clinical and anatomical characteristics, we investigated the influence of risk factors on the occurrence and evolution of BSC carcinomas. These factors include patient age, lesion size, and the degree of sun exposure. Our analysis also included aspects related to changes observed in peripheral blood in an attempt to identify possible correlations between the patients' hematological profile and the aggressive nature of this skin cancer type.

Our findings, highlighted in this detailed analysis, raised important questions and emphasized the necessity of a deeper approach to basosquamous carcinomas in clinical practice. Additionally, the absence of a standardized treatment underscores the need for further research to develop personalized and effective therapeutic strategies for patients affected by this particular form of skin cancer.

The association between carcinoma type (basosquamous or squamous) and changes in inflammatory markers such as ESR, NLR, and LMR is an interesting aspect in the context of sun exposure and skin cancer evolution.

An elevated ESR can indicate the presence of an inflammatory reaction in the body. This aspect can be associated with inflammatory processes induced by sun exposure, especially since CSC is often linked to ultraviolet radiation exposure. Persistent inflammation can contribute to the evolution and aggressiveness of CSC.

A higher neutrophil/lymphocyte ratio may indicate an inflammatory reaction and possible immune system suppression. This could result from the immune response to high sun exposure, which in CSC can contribute to tumor progression and development.

A higher lymphocyte/monocyte ratio may suggest improved immune activity or a specific inflammatory approach. This aspect may be relevant in the context of BSC, which,

although associated with sun exposure, may have a more benign profile and slower evolution than CSC.

Although there are no studies in the literature addressing exactly non-melanocytic skin cancers, a study showed that an NLR greater than 5 had a significantly poorer prognosis for overall melanoma survival and was associated with a significantly lower progression-free survival rate, as well as an NLR cut-off greater than or equal to 3, but this did not apply to an NLR less than 3 [36].

The association of these changes with high sun exposure is remarkable and consistent with the literature indicating that excessive ultraviolet radiation exposure can significantly contribute to CSC development. It is important to mention that these associations do not necessarily represent causal relationships but can provide valuable clues for understanding the interactions between sun exposure, inflammation, and specific types of skin cancer.

In general, these observations underline the complexity of interactions between environmental factors, immune response, and skin cancer evolution, highlighting the importance of further investigations and research to clarify these relationships and contribute to developing more effective therapeutic strategies.

Conclusions

Clinical activity revealed a significantly increased incidence of basosquamous carcinomas compared to the figures reported in the literature. This discrepancy suggests possible local particularities or specificities in the prevalence of BSC and emphasizes the importance of continuous investigation to better understand the distribution and frequency of this skin cancer type.

BSC presents complex and controversial characteristics, combining features of basal cell carcinoma and squamous cell carcinoma. The absence of a standardized treatment poses a challenge in managing this condition, underscoring the necessity of further research to develop personalized and effective therapeutic strategies to improve prognosis and optimize clinical management.

Detailed analysis of variables such as patient age, lesion size, sun exposure, and hematological changes revealed a significant association between high sun exposure and changes in inflammatory markers. This correlation brings into discussion the possible influence of excessive ultraviolet radiation exposure on skin cancer evolution, opening the way for

further research to clarify these relationships and contribute to optimizing prevention and treatment strategies.

Comparing BSC and CSC recorded statistically significant differences in LY%, NLR, LMR, and ESR, which may suggest a different inflammatory and immune response.

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