IOSUD – UNIVERSITATEA „DUNĂREA DE JOS” DIN GALAȚI

Școala doctorală de Științe Biomedicale

TEZĂ DE DOCTORAT

BASAL CELL CARCINOMA MORPHOMETRIC ANALYSIS WITH FOCUS ON PERITUMORAL CLEFTING PHENOMENA

Doctorand,

Badiu (Niculeț) Elena

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Seria M Nr. 2

GALAŢI

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LIST OF SYMBOLS AND ABBREVIATIONS

BCC – basal cell carcinoma

BC – before Christ

WHO – World Health Organization

NMSC – non-melanoma skin cancer

UV – ultraviolet

PUVA – psoralen and ultraviolet A

RCM – reflectance confocal microscopy

OCT – optical coherence tomography

sBCC – superficial basal cell carcinoma

nsBCC – non-superficial basal cell carcinoma

MMS – Mohs micrographic surgery

nBCC – nodular basal cell carcinoma

pBCC – pigmented basal cell carcinoma

mBCC – micronodular basal cell carcinoma

iBCC – infiltrative basal cell carcinoma

mfBCC – mopheaform basal cell carcinoma

bsqBCC – basosquamous basal cell carcinoma

SCC – squamous cell carcinoma

smBCC – superficial multicentric basal cell carcinoma

SARS-COV-2 - Severe Acute Sespiratory Syndrome COronavirus-2

COVID-19 – COronaVirus Disease-2019

HCV – hepatitis „C” virus

IgE – immunoglobulin E

IL-7 – interleukin-7

IL-23 – interleukin-23

INTRODUCTION

Basal cell carcinoma (BCC) is one of the most common malignant skin tumors found on the sun-exposed areas of elderly patients, developing from the basal cells of the interfollicular epidermis or the hair follicle epithelium. Ther current rise in BCC numbers among younger patients is possibly due to personal habits and environmental exposure.

The current study aimed at working on a general and particular morphometric evaluation of BCCs and the specific phenomena of tumor-stroma clefting, the separation that takes place between the tumor nodules and their surrounding stroma. Once considered an artifactual retraction space due to the pathology laboratory tissue processing, due to new in vivo investigation techniques (reflectance confocal microscopy, optical coherence tomography) this phenomenon was identified as being present in BCC tumors, in living tissue, as a tumor trait. This study had the main aim of identifying the significance of the cleft and the possible consequences that its presence might have on the tumor and patient.

DOCUMENTING STAGE

CHAPTER 1 – Epidemiological data and etiopathogenic aspects

1.1 Basal cell carcinoma history. The history of medicine describing cancer as an entity dates back to the Egyptians, in the year 2500 before Christ (BC), followed by Hippocrates who coined the term "carcinos" for malignant tumors. BCC was initially described in 1827, but Krompecher (in 1900) made its description as a malignant skin tumor and gave it the name "Basalzellenkrebs". The World Health Organization (WHO) maintains the name "basal cell carcinoma" since 1974. [1,2]

1.2. Basal cell carcinoma epidemiology. BCC presents a multifactorial etiology involving multiple intrinsic and extrinsic factors, with a slow growth rate, extremely low metastatic potential and mortality rates. The true incidence and prevalence of BCC cases are uncertain, but in 2006, the American Cancer Society reported that over 2 million people were receiving treatment for non-melanoma skin cancer (NMSC, most of them being a BCC type). In 2016, the BCC incidence was on the rise (at a rate of approximately 3 to 8% per year), especially in the young, female population. [3-11]

1.3. Basal cell carcinoma etiopathology. BCC development involves multiple factors classified into intrinsic (patient-dependent) and extrinsic ones (external). [12] Some patient-dependent factors are: individual constitutional traits – the patient's skin type (fair-skinned individuals), presence of nevi, longevity, immune suppression, scars, hereditary disorders (xeroderma pigmentosum, nevoid BCC

syndrome), preexistent cutaneous lesions (eczema) and previous BCC. Some extrinsic factors are: increased natural or artificial ultraviolet light (UV) exposure, X-ray exposure, seasonal or outdoor work during the summertime, occupational chemical exposure, exposure to psoralen and ultraviolet A (PUVA) therapy, retinoids, tetracycline. [3,6,7,9,11]

CHAPTER 2 – Clinical/gross aspects of basal cell carcinoma. BCC is a slow-growing tumor with low metastatic potential and favorable prognosis. [13] It develops on sun-exposed areas of the body, most frequently found on the head and neck region more specifically on the nasal pyramid area. [7] Rare locations for developing such a tumor are considered to be the genital and perianal regions, the palm and soles, and the nail unit. [14,15] The clinical appearance of a BCC varies widely according to its multiple subtypes. The BCC's classical clinical features are that of a telangiectatic, pearly papule with erosion and/ulceration, [15] varying towards erythematous patches or large, ulcerated nodules with local invasion of the surrounding structures and destruction, or even pigmented and non-pigmented lesions. [16]

CHAPTER 3 – Non-invasive diagnostic tools for basal cell carcinoma. BCC diagnosis is mainly a clinical one, with aid from multiple techniques such as dermoscopy, reflectance confocal microscopy (RCM) or optical coherence tomography (OCT), and histopathology. [12]

3.1. Dermoscopy. Dermoscopy, is an in vivo surface microscopy (allowing skin structure analysis from the epidermis to the superficial dermis) at 10-fold optical magnification, improving diagnostic accuracy. BCCs are characterized dermoscopically by a set of main criteria: ramified vessels (fine/short), focused dots, large, blue-gray, ovoid/round globules/nests, leaf-like/spoke-wheel areas, pink-white areas and/or short, white streaks, ulceration. [13,17-19]

3.2. Reflectance confocal microscopy. RCM allows the rapid scanning of a patient's lesion, with en face sections with 30 times magnification; it evaluates a BCC's lateral margin extension prior to surgical intervention. BCCs are revealed on RCM by many parameters: solid tumor islands with a less bright image (compared to the surrounding stroma), made up from cells with elongated nuclei (with peripheral palisading), tumor-stroma separation spaces – peripheral dark areas. [16,17,20,21]

3.3. Optical coherence tomography. OCT uses infrared light in order to study BCC. The tumor islands located at epidermal or upper dermal levels are viewed as oval dark/hyporeflective silhouettes, surrounded by a dark border, with/without a hyper-reflective stroma around them. The central cystic degeneration/necrotic cavities of the tumor lobules are areflective, round structures. [18,23-26]

3.4. Combined reflectance confocal microscopy-optical coherence tomography evaluation. This RCM-OCT combined technique aids the physician in making a three-dimensional analysis of the

patient's lesion, with cross-sectional images from OCT and en face images from RCM. [18]

3.5. Exfoliative cytology. It is a rarely used method of evaluation in dermatology because it is not able to differentiate between tumor types. Pasquali et al. (2020) revealed differences between superficial BCC (sBCC) and other BCC subtypes (non-superficial BCC (nsBCC)) in exfoliative cytology samples, with moderate cellularity, mild atypia and dehiscence characterising sBCC, while high cellularity, severe atypia and dehiscence indicating nsBCC. [27]

CHAPTER 4 – Histopathology of basal cell carcinoma. A BCC diagnosis can be made by non-invasive or invasive techniques (shave or punch biopsies, surgical excision - classical or Mohs micrographic surgery – MMS). Histopathological examination classifies BCC in two categories of risk of recurrence. As such, WHO reveals that low-risk tumors are considered to be: sBCC, nodular (nBCC), pigmented (pBCC), infundibulocystic and the Pinkus tumor; high-risk BCCs are: micronodular BCC (mBCC), infiltrative (iBCC), morpheform (mfBCC), basosquamous (bsqBCC) and BCC with sarcomatoid differentiation. [15] As a general aspect of such tumor, BCCs are seen microscopically as consisting of different-sized nodules/islands of basaloid-looking cells, equivalent to the basal cells located in the first layer of the interfollicular epidermis, and the squamous epithelium located in the pilosebaceous unit. [17,28] The tumor islands feature a peripheral rim of palisading cells (with hyperchromatic, large nuclei, little cytoplasm) with central apoptotic cells, melanocytes and/or keratin, and a separation area or detachment space located in-between the tumor islands and the stroma surrounding them, the latter being regarded up until recently as being an artifactual entity, developing in the course of histopathology laboratory processing. [15,17,28,29] The changes of the stromal element of a BCC result in a fibro-myxoid stromal component, with or without amyloid deposits. The classical histopathology subtyping is well documented, with highlighted specific traits for each BCC, by the WHO in their latest 2018 "Classification of skin tumors", 4th edition, revealing the subtypes: sBCC, nBCC, mBCC, iBCC, mfBCC, bsqBCC, pigmented, with sarcomatoid differentiation, with adnexal differentiation, and fibroepithelial BCC. [15]

CHAPTER 5 – Histopathological differential diagnosis of basal cell carcinoma. The histopathological differential diagnosis of BCCs includes especially tumors with basaloid or follicular differentiation (trichoepitelioma, basaloid squamous cell carcinoma – SCC). [15] sBCCs are differentiated from actinic keratosis, mfBCCs from desmoplastic trichoepitelioma and microcystic adnexal carcinoma. nBCCs should be differentiated from trichoblastic neoplasms, Merkel cell carcinoma, adnexal tumors (adenoid cystic carcinoma). mBCC can be differentiated from nBCC with a mBCC deep component. BCC with sebaceous differentiation should be differentiated from

sebaceous tumors, while BCC with ductal differentiation from sweat gland carcinomas. bsqBCC is distinguished from keratotic BCC, collision between SCC and BCC, and benign pseudocarcinomatous epidermal hyperplasia. Sarcomatoid BCC is differentiated from BCC with myoepithelial differentiation, collision between BCC and atypical fibroxanthoma/undifferentiated pleomorphic sarcoma, cutaneous leiomyosarcoma or osteosarcoma. Pinkus tumor should be differentiated from eccrine syringofibroadenoma. [15,30]

CHAPTER 6 – Basal cell carcinoma treatment options. BCCs benefit from a myriad of (local) treatment options, such as: surgical excision, MMS, diathermocoagulation, curettage, cryotherapy, photodynamic therapy, topical cytotoxic drugs – imiquimod, intralesional chemotherapy, radiation therapy, laser therapy. [3,7,17,31] The preferred BCC treatment is represented by a complete surgical excision, with histological assessment of the surgical margins. [32] Complete excision must be made with the proper safety margins of at least 4 millimeters of tumor-free tissue (for low-risk tumors) and at least 6 millimeters for high-risk tumors. [9,33] Surgical excision of the tumor by MMS is considered the best treatment choice; it involves making numerous serial excisions in order to obtain free tumor margins, these serial tissue fragments being examined by frozen section technique. It is most frequently used in high risk tumors, having higher rates for long-term cure. [22,34] The recurrence rates of BCC are variable and influenced by: the initial treatment strategy, positive deep surgical resection margin, the incomplete surgical excision (with rates ranging from 4 to almost 17%), the histopathological traits (especially the presence of certain BCC subtypes – such as: bsqBCC, mfBCC or pleomorphic), the BCC's development site, and/or by the presence of the inflammatory infiltrate surrounding the tumor mass. Recurrence seems to develop with higher frequency on the head and neck regions. [7]

PERSONAL CONTRIBUTIONS

CHAPTER 7 – Material and methods. The current work consists of a study which aimed to work on a general and particular morphometric evaluation of BCC, with focus on their specific phenomena of tumor-stroma clefting. Patient group characteristics and BCC tumor traits were analyzed (including the tumor's Clark level, lymphocytic reaction, perineural invasion), along with measurements that were made in regards to the BCC mass: the overall maximum tumor dimension, the tumor's nests, the maximum blood vessel diameter surrounding the tumor mass. Emphasis was put on the clefting phenomena by measuring the largest cleft's width, its corresponding tumor nest dimension, the presence or absence of a material at this level, having as main aim to discover the cleft's significance

and what consequences might its presence bring on the tumor and patient (prognosis). The current study is a retrospective one which included a number of 244 patients having histopathological confirmation of BCC diagnosis, extended on a span of 2 consecutive years, starting from January 1st 2019 and up to December 31st 2020. This research was carried out at the „Sfântul Apostol Andrei" Emergency Clinical Hospital of Galați, Romania, in the Pathology Laboratory, on cases received from the hospital's clinics and out of hospital cases that addressed the Pathology Laboratory for surgical sample analysis. The confirmed BCC cases' analysis included materials found in the hospital's electronic database, the pathology laboratory's documents and the tissue-prepared slides found in the histopathology slide archive of the aforementioned Pathology Laboratory.

The inclusion criteria for this study consisted of: skin tumor-patients positively diagnosed during 2019 and 2020 having a histopathological BCC diagnosis, after making a revision of the patients' tissue slides and confirming their diagnosis. The exclusion criteria for the current work consisted of: histopathology-studied cases (diagnosed from 2019 to 2020) with other pathology, cases which at the diagnostic revision step proved to be unconfirmed cases, not meeting the BCC positive diagnosis criteria.

The following parameters were analyzed: clinical, epidemiological and histopathological ones, and these general objectives were established: (1) The overall extension of the knowledge on BCC clinical signs, epidemiological traits and histopathological aspects with morphometric analysis; (2) The devise and creation of an electronic database which includes data regarding epidemiological, clinical and histopathological parameters of the patients included in the study group; (3) The general morphometric analysis of BCC tumors; (4) An in-depth morphometric analysis of the tumor-stroma cleft phenomena, with further correlation with other BCC and patient characteristics.

The methods used in order to reach these specific objectives included: (1) the clinical and epidemiological analysis for the selected patients: age, gender, clinical aspects, tumor location, tumor dimensions; (2) a histopathological and morphometric analysis of the BCC cases in order to establish the BCC subtype and tumor recurrence risk; (3) the statistical analysis which aides in establishing significant correlations between the parameters evaluated.

CHAPTER 8 – Basal cell carcinoma morphometric analysis with focus on peritumoral clefting phenomena. The current work includes a number of 106 tables and 86 figures.

8.1. Patient group. The current work included 244 patients with a M:F sex ratio of 1.03,

most patients addressing health care services in 2019 (67.6%). The patient group includes mostly adult and elderly patients 60-89 years of age (85.2%); younger patients were male, while elderly ones were more frequently females. Most patients (67.2%) came from the urban environment, were retired (86.9%) and had no allergies (97.13%). The patients presented multiple associated pathologies (comorbidities) of various organ systems, the most frequent being: cardiovascular diseases (52.9%), male genital system pathologies (16.9%) and other diseases (diabetes, anxiety, depression - 18.4%), mostly in the elderly population (statistically significant). The patients suffered from various other types of non-cutaneous tumors (4.5%), either malignant or benign - colon adenoma, dermatofibrosarcoma protuberans, meningioma, non-Hodgkin lymphoma, lipoma.

8.2. Basal cell carcinoma study. An increasing number of patients suffering from associated skin pathology was found, an ascending trend proportional with the patient's age ($p = 0.023$), almost half having 70-79 years of age (45.3%). Fifty-seven percent of 70-79 years-old patients presented as a total number of 1 skin lesion/person, but more than 1/4 of them have 2 lesions (25.6%) in total (benign or malignant); 68.1% of patients of over 80 years also registered 1 skin lesion and 15.3% of them had 2 skin lesions in total; in patients over 70 years, the percentages of cases with a total number of 3 or 4 skin lesions were found to be also slightly increased; all with statistical significance ($p = 0.036$).

The most frequent associated skin pathology was seborrheic keratosis (30.8%) and acrochordon (23.1%), but other lesions were also found with relatively high incidences – SCC (19.2%), epidermoid cyst (15.4%), melanocytic nevus (17.3%). SCC was much more frequently found in males (29.0%), as compared to females (4.8%). The number of malignant skin lesions per person was found to increase with age, > 90% of patients under 70 years having 1 malignant lesion, while 19.8% of 70-79 year-olds and 12.3% of >80 year-olds had 2 (or at least 3, in fewer cases), ($p = 0.045$), revealing a relationship between the patients' age and the number of malignant skin lesions.

In regards to the presence of associated BCCs: those over 70 years had associated BCCs in more than 60% of cases. The number of associated BCCs increases with age, patients with 2, 3 or even ≥4 lesions having over 70 years (20.9%, 5.8%, 4.7%) and 80 years, respectively (12.3%, 1.4%, 6.8%), ($p = 0.004$), revealing a relationship between the patients' age and the total number of BCC lesions.

Thirty patients were diagnosed with prior BCC (1-5 lesions) - Figure 1, with no association with tumor subtype, but multiple prior BCCs were found more frequently in

mfBCC, sBCC and Pinkus tumor cases (5 prior lesions) and 3 or 4 prior BCCs were recorded in patients with nBCCs and superficial multicentric BCC – smBCC, (p = 0.000).

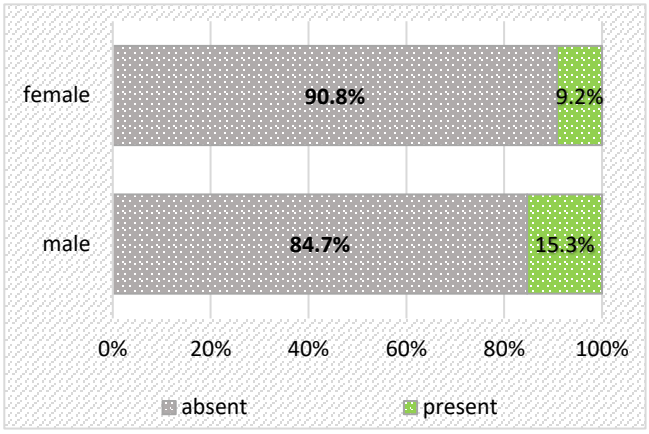

Figure 1. Prior BCC diagnosed patients by gender

Six patients developed subsequent BCCs (following the BCC diagnosis taken into account in the study, they developed other BCCs), most with 1 lesion and 1 patient with 14 lesions. It is associated with the BCC subtype (p = 0.041) initially diagnosed in the current study, being identified in cases of iBCC (13.6%), mBCC (6.7%), and nBCC (1.1%).

The positive clinical diagnosis of BCC was made only in 18.4% of cases, physicians clinically suspecting various other types of lesions (malignant melanoma, SCC, nevi or even molluscum pendulum).

The most frequent subtype of BCC found in our patient group was the nBCC (74.2%); also frequent was iBCC (9.0%). nBCC was more frequently discovered in females (77.5%, vs. 71.0% of men), while the iBCC occurred more frequently in males (12.1% vs. 5.8% females), (Chi2 = 14.252, p = 0.047). bsqBCC subtype was detected more frequently in males (4.0% vs. 0.8% females), while smBCC was more frequent in females (7.5% vs. 2.4% in males).

The iBCC was mainly associated with other infiltrative (42.9%) or nodular (28.6%) subtypes, while the mBCC was mainly associated also with micronodular cases (50.0%), as well as the nBCC (71.0%). 1 patient with sBCC has associated also a superficial one and cases with smBCCs are associated mainly with other nBCCs (60.0%); (p = 0.000).

The BCCs were found most frequently at the level of the forehead (18.4%), the nasal pyramid (18.4%), cheek (12.7%) and, the eyelid (9.8%), with no differences among genders.

Solar elastosis was found in almost all BCC cases (91.8%). Tumors that associated solar elastosis were located most frequently on the cheek (13.4%), the forehead (19.2%),

nasal pyramid (19.6%) and, respectively, the eyelid (10.3%), locations which also associated tumor ulceration; tumors without solar elastosis were located more frequently on the auricle (15.0%) and the posterior thorax (15.0%); (p = 0.000).

Patients with tumor ulceration suffered most frequently from iBCCs (10.9%) and mBCCs (7.3%), while the sBCCs and smBCCs had no ulceration (p = 0.000). All patients with bsqBCC, mBCC, mfBCC and smBCCs had solar elastosis, while the sBCCs, iBCCs and Pinkus tumor did not (p = 0.012).

The most frequent BCC subtype found in glabrous skin tumors was nBCC (78.7%) or iBCC (10.1%), while for non-glabrous skin located tumors the most frequent one was also nBCC (58.9%), as well as smBCC (19.6%), (p = 0.000). Patients with glabrous skin located tumors had significantly smaller tumors (with reduced maximum tumor dimensions, 9207.577 ± 6083.5622) as compared to non-glabrous skin located tumors (14491.464 ± 12089.3448).

bsqBCC was located at the level of the auricle, forehead, nasal pyramid, neck, scalp and posterior thorax, while the iBCC at the level of the nasal pyramid (27.3%) and forehead (22.7%), as well as for mBCC cases (26.7%, respectively 20.0%), (p = 0.000).

The largest tumor dimensions were found in tumors located on the forearm (30120.000), the calf (26281.250 ± 17378.9169) and the anterior thorax (20602.250 ± 29529.6203), respectively; the smallest ones were found at the level of the posterior abdomen (5336.067 ± 4489.8693), as well as on the anterior abdomen (6797.500 ± 7463.5121) and on the lip commissure (6789.9460 ± 4190.1725), (p = 0.000).

The largest tumor dimension was found to be influenced by the patient's age (p = 0.003) and BCC subtype (p = 0.015), and had the tendency to grow as long as the patients' age increased as well, larger tumors being found in bsqBCCs (14934.417 ± 6705.5960); it was also directly proportional to the largest tumor nest's dimensions (r = 0.250).

The largest tumor nest's dimensions were found in nBCCs (1808.309 ± 1519.4810) and bsqBCCs (1780.933 ± 817.7539), while the smallest ones were reported in sBCCs (267.367 ± 112.6863), (p = 0.000).

Most patients had tumor Clark levels of IV (56.1%) or even V (30.7%). Clark level II tumors characterized smBCCs (63.6%), while iBCCs had Clark level IV (10.2%) or V (10.7%), (p = 0.000). Younger patients (under 60 years) were found to have Clark level II or III tumors, while elderly patients had deeper, Clark level tumors (IV or V), (p = 0.028). Males had mostly tumors with Clark level IV (62.9%) (p = 0.001). The largest tumor nests

had the smallest values in Clark level II tumors (1144.345 ± 2527.8942), while the largest ones were found in Clark level III tumors (1919.476 ± 2005.1779), (p = 0.023).

The largest tumor nest's measurements were also increased in regards to the body's poor lymphoid reaction to the tumor mass (1821.870 ± 1704.2468), as opposed to the rich lymphoid reaction (1246.520 ± 1081.6276), (p = 0.008).

The tumor thickness varied between 12.0 and 50620.0 μm. The largest tumor thickness was found in iBCCs (3896.500 ± 10479.7479 μm), while the smallest one was found in sBCCs (473.167 ± 261.8269 μm), (p = 0.000); it was also significantly correlated with the largest tumor dimension (r = 0.256), being directly proportional.

Statistically significant differences were found between cases with follicular differentiation and the BCCs' recurrence risk (p = 0.046); most cases with follicular differentiation (34.0%) were identified in low risk tumors (having a high risk component), decreasing in low risk BCCs (18.1%). Squamous differentiation was most frequently found in bsqBCCs (66.7%), while in other BCC types it was rarely found (p = 0.001).

Half of the tumors in the studied group (49.2%) did not have perineural invasion; in the other half, 20.1% of patients had perineural invasion and the other 30.7% had suggestive signs of perineural invasion (perineural chronic inflammation). Perineural invasion was found in older patients (p = 0.000); it was identified in 14.3% of 40-49 year-old patients, while 54.6% of 70-79 year-olds (and 70.8% of 80-89 year-olds) had perineural invasion or suggestive signs thereof. The tumor measurements were also significantly larger (p = 0.000) in tumors with perineural invasion (13683.965 ± 8791.6125) or suggestive signs thereof (11418.315 ± 10066.0127), as opposed to those without perineural invasion. Only 14.3% of Clark level III tumors had perineural invasion, while 37.2% level IV and 54.7% level V tumors showed perineural invasion (p = 0.000). Perineural invasion was found in all bsqBCCs and mfBCCs, in most iBCCs or mBCCs and in 44.8% of nBCCs, being absent in Pinkus or sBCCs. Most patients with low risk tumors did not have perineural invasion (66.7%), while over 80% of those with high risk tumors or high risk with low risk component presented with perineural invasion or suggestive signs thereof (p = 0.000).

Patients without allergies had poor and rich lymphoid reaction to the tumor mass in similar percentages, while those with allergies had mostly poor lymphoid reaction (71.4%).

The blood vessel's maximum diameter was evaluated by measuring the diameter of the blood vessels located in the close vicinity of the tumor mass (approximately 0.2-0.3 mm). The blood vessels' maximum diameters varied between 13.2 ÷ 580.0 μm, being increased in the elderly (maximum diameters in 80-89 year-olds (122.991 ± 86.6185)), (p = 0.035).

The largest diameters were recorded in cases of bsqBCC (138.183 ± 71.7028) and nBCC (112.150 ± 86.3560), while the smallest one was recorded in sBCCs (24.900 ± 6.7022). The largest tumor dimension and, respectively, the largest tumor nest's dimension and the blood vessel's maximum diameter (r = 0.367) are directly proportional. Slightly larger blood vessel maximum diameters were found in patients having solar elastosis (104.300 ± 80.0856), poor lymphoid reaction (109.870 ± 84.4981), (p = 0.351), perineural invasion (118.414 ± 81.7994) or suggestive signs thereof (114.371 ± 95.2075), (p = 0.046), Clark level 3 (119.910 ± 110.4857) and Clark level 5 (117.976 ± 79.5553), (p = 0.001).

BCC recurrence risk stratification separated tumors into 2 categories : low risk and high risk. However, there are cases where high risk tumors associate a low risk component and vice-versa. The current study highlighted 4 main BCC categories, the most frequent ones being low risk BCCs (81.1% - of these, 21.8% associating a high risk component). The BCC recurrence risk was not associated with the patient's age, nor gender.

8.3. Basal cell carcinoma's tumor-stroma cleft phenomena study. The average value of the maximum width of the cleft was 48.136 ± 54.7362 μm, with a range of variation between 5.5 ÷ 511.0 μm. The cleft's corresponding tumor nest's width had an average value of 952.587 ± 1174.6217 μm, with a range of 29.0 ÷ 8500.0 μm. The largest clefts were found in nBCCs (54.691 ± 60.2684), while the smallest ones in Pinkus tumors (10.500), (p = 0.000). The largets cleft's corresponding tumor nest widths were recorded again for nBCCs (1154.809 ± 1280.9217); the smallest one were reported in Pinkus tumor (113.600), (p = 0.000). The proportion between the cleft's corresponding tumor nest's width and the maximum cleft's width itself recorded values between 1.04 – 317.77, this ratio being different among BCC subtypes (p = 0.022) - the largest ones being found nBCC (32.6966 ± 44.37777), while the smallest ones were reported for smBCC (9.8158 ± 6.79144).

The average value of the largest tumor nest's cleft width was found to be 36.401 ± 48.8326 μm, which was linked to the BCC subtype (p = 0.001) - the largest values were found in nBCCs (41.861 ± 53.8424), while the smallest ones in Pinkus tumor (6.700).

Tumor nest cleft development was found in all cases of BCC. The cleft was also found in all BCC components in 89.3% of cases and in all tumor nests in 48.4%. The largest tumor dimension between the samples defined by cleft development in BCC components was not statistically significant (p = 0.294). The other tumor measurements (cleft's maximum width, cleft's corresponding tumor nest width, largest tumor nest's cleft width, largest tumor nest dimension) were instead significantly larger, almost doubled in cases with

cleft present in all tumor nests, as compared to the others.

The cleft was present in all tumor nests in all sBCC cases, in over ½ of smBCCs and nBCCs and in ¼ of mBCCs ($p = 0.000$). The number of cases with cleft development in all tumor nests much higher in cases with optically clear clefts (56.8%) than for clefts with bluish material (39.5%). The latter was observed more frequently in cases with larger tumor dimensions ($p = 0.203$), larger tumor nests ($p = 0.504$), follicular differentiation ($p = 0.201$), and cases of iBCC (11.8%) and the optically clear cleft was observed in cases of smBCC (8.0%), while nBCC had almost equal ratios ($p = 0.064$).

The cleft's maximum width was found to be increased in tumors with follicular differentiation (63.031 ± 88.0315); this is also valid for the largest tumor nest's cleft width (46.782 ± 76.9381); it is also directly proportional with the tumor thickness ($r = 0.162$).

The patients having tumors with squamous differentiation had larger clefts (49.035 ± 56.5480). The cleft's maximum width was found to be almost doubled in cases with optically clear clefts (60.222 ± 69.9812), as compared to measurements found in cases with bluish material located in the cleft (35.441 ± 26.5742), ($p = 0.002$).

The cleft's maximum width was significantly higher in low risk BCCs (51.833 ± 57.3756) and in low risk with high risk component (54.743 ± 62.3824), being almost doubled compared with the other categories ($p = 0.001$). The largest cleft's corresponding tumor nest width were recorded in low risk BCCs (1230.944 ± 1402.1562), while the smallest ones were recorded in high risk cases (378.256 ± 427.1651), ($p = 0.000$). Slightly increased cleft dimensions were found in patients with solar elastosis (48.242 ± 54.2323).

The cleft's maximum width was the smallest in Clark level II tumors (29.036 ± 13.4868), being increased for other Clark levels, and particularly for the Clark level III tumors, where its average size was 60.019 ± 106.4750. The Clark level was correlated with the cleft's corresponding tumor nest width ($p = 0.002$) - very small nests in level II tumors (233.864 ± 164.0277) and maximally increased for level III (1454.024 ± 1903.4737).

The cleft's maximum width was higher in cases with poor lymphoid reaction to the tumor mass (54.519 ± 68.5101), as opposed to cases with rich lymphoid reaction (42.446 ± 37.9250); this was also valid for the cleft's corresponding tumor nest width (difference between categories was much larger) - the average size was 1182.420 ± 1467.5093 in poor lymphoid reaction vs. 747.698 ± 782.0113 in rich lymphoid reaction cases.

Patients having BCCs with perineural invasion or suggestive signs thereof had smaller cleft maximum widths, corresponding tumor nest widths and smaller tumor nests, these differences being statistically significant only for the cleft's corresponding tumor nest

width (p = 0.032). The cleft's maximum width was significantly correlated with the blood vessel's maximum diameter, as well as the cleft's corresponding tumor nest width.

CHAPTER 9 – Discussions.

9.1. Patient group. The current study revealed a peculiar finding of a predominance of health care services addressability in the year 2019, as opposed to 2020 (almost 2/3, versus 1/3 of patients), possibly explained by the emergence (in 2019) with rapid contagiousness and extension of the Severe Acute Respiratory Syndrome COronaVirus 2 (SARS-COV-2) infection, accountable for the Coronavirus disease 2019 (COVID-19), a pandemic state which lowered the number of patients who sought medical care (Núñez, Sreeganga and Ramaprasad (2021); Gertz, Pollack, Schultheiss and Brownstein (2022); Khalawany et al. (2022) and Cocuz et al. (2022)). [35-38]

The patient group's structure in regards to patient age reflects findings from the medical literature data, BCC being frequently found in patients of 65 years and older [39,40], but with a rising incidence in patients over 40; [41] also, younger patients were males, in contrast with the medical literature which reports higher incidences of BCC among males over 65 [42-44] (testifying that male sex might be a fundamental patient characteristic which submits them to the risk for BCC development). [37,42,44]

The results regarding patient origin environment (the majority of patients came from the urban environment) are in accordance with Cocuz et al. (2022), reporting a higher incidence among people from the urban areas [38], and not from the rural ones, as medical literature reported in the past. [45-47] The finding regarding the allergic status of the patients – most of them being non-allergic, sums up the inverse relation which BCC development has with the allergy status of such patients. The supporting role of IgE (immunoglobulin E) in mediating the immune system's response to the neoplastic process, in allergic patients, decreases the body's capacity to fight neoplasia. [48]

The current study also reports the associated pathologies/comorbidities that BCC patients suffer from, in accordance with the current medical literature - cardiovascular comorbidities (such as atrial fibrillation, hypertension, ischemic heart disease, cardiac insufficiency), central nervous system diseases (dementia), chronic hepatitis "C" virus (HCV) infection, type II diabetes, obesity and depression, findings which support the overall patient characteristics from our group and which are nevertheless a reflection of the majority of elderly patients which took part in our study, [49-53] with implication in patient treatment, their management being more complicated. Conventional treatments are reported to be less

successful (surgical approach, radiation therapy, photodynamic therapy local therapeutic agent use). Even more so, the association between BCC and comorbidities that are related to immune suppression (associated cancers, chronic HCV and hepatitis "B" virus infection, [54-56] or other immune suppressive states) reveal an organism's state of photosensitization, oncogenic effects and impaired or defective immune system surveillance (which provides the basis for unrestricted development and progression of cancer cells). [54]

Literature data considers that BCC (but also SCC) are not in fact deadly malignant tumors and comorbidities might precipitate the patient's exitus, [50] being mortality predictors and an increasing treatment burden, having a significant impact on the patient's functional reserve. [51,57]

9.2. Basal cell carcinoma study. The current research reported on the skin lesions that are associated with BCCs, inflammatory or neoplastic in nature - SCC and its preinvasive precursors (some patients possibly being at a higher risk for developing NMSC, actinic keratosis being a sign of actinic damage), dysplastic nevus, epidermoid and trichilemmal cyst, scars, chronic dermatitis – psoriasis vulgaris, associations that might be coincidental, but also a possible secondary development due to a Koebner phenomenon subsequently to trauma, or a Wolf (post-herpetic) isotopic response in an already immune suppressed skin area, or the result of epidermis and dermal adnexa atrophy with sensitivity to UV radiation. [58] There are factors which seem to predispose an individual with psoriasis to carcinogenesis, such as: PUVA therapy in high doses (a type of photochemotherapy), [59,60] comorbidities, personal habits, immune suppressive (non-biologic) treatment, chronic inflammation [59] (with inflammatory mediators involved in both diseases (interleukins 7 and 23 (IL-7, IL-23), inducing adaptive responses and pro-cancerous mutations, increase apoptosis resistance and stimulate neoangiogenesis [60]).

This research concluded that as an associated BCC lesion, SCC was more frequently found in males. The current literature reports that men had a higher incidence of both BCC and SCC, possibly due to working outdoors (either as recreation or work-related), and even more so they develop a second NMSC or even multiple tumors. [61,62]

The patients included in our study are mostly elderly ones, some of them having multiple skin lesions (one with a total of 18 lesions). BCC patients were found to have increased risk of developing other BCCs, a patient registering the presence of 17 tumours (most probably a syndrome affiliation). At the time of the main tumour's diagnosis, 12,3% of BCC patients had prior BCCs, while 2,5% subsequently developed BCCs.

A statistically significant element was found in regards to the subtype of BCC (as primary tumour diagnosis) and the number of prior BCCs: nBCC, iBCC and smBCC registered the highest number of prior tumours, while nBCC and iBCC are the most frequent BCC subtypes most likely to be associated with subsequent BCC development, mostly in men.

A a positive diagnosis of BCC was suspected only in 18,4% of cases, physicians clinically diagnosing other types of lesions such as malignant melanoma, SCC, melanocytic nevi or molluscum pendulum, in accordance with literature data. [41,63-66] The data regarding the frequency of BCC subtypes found and the gender possibly preferential involvement reflects a proclivity in men towards developing BCCs, and more importantly high-risk tumors (such as bsqBCC and iBCC). [67-71] Just as it was registered in our study, certain BCC subtypes offered the risk of developing the same subtype in the future: iBCC is a risk for developing iBCC, similar for bsqBCC, while the nBCC tends to be a risk for developing nBCC or sBCC. [72]

Derebaşınlıoğlu and Özkaya (2021) report that the nasal pyramid is the most frequently affected site of the face (and body), in accordance with our findings, a reason for this finding possibly being the fact that this area is difficult to protect against UV radiation. [73] Karlin et al. (2020) report similar findings in regards to the gender distribution of BCC's location, men having more frequently tumours on the auricle and scalp, [74,75] while female patients registered as frequent locations the chin and nasal pyramid. [74] These data suggest a gender specific pattern of sun exposure, and possibly different approaches to sun protection, with subsequent solar elastosis development as a response to UV radiation (mainly involving the nasal pyramid and forehead), with sun protected areas being far less involved by such process.

Yalcin et al. (2015) report the presence of ulceration as a more frequent finding in high risk, aggressive tumors, such as: iBCC, mfBCC, mBCC, bsqBCC [76] which is similar to our findings. Urech et al. (2017) researched whether ulceration might have a positive outcome implication and have found that ulceration indicates a more positive response of a BCC to topical treatment (with imiquimod). [77]

Regarding UV damage to the skin's structure, solar elastosis was found among the patient's in the group in those with high risk BCCs - bsqBCCs, mBCC, mfBCC. Lesack and Naugler (2012) found correlations between the BCC subtype and solar elastosis presence, being more commonly found in iBCC, nBCC and sBCC, linking the effective sun damage to the tumour's phenotype. [78]

The current study has found that BCCs have a tendency to develop in certain locations according to subtype. Aggressive, high-risk tumors (bsqBCC, iBCC, mBCC, mfBCC) were found on frequently exposed areas to the UV light: forehead and the nasal pyramid, but also auricle and neck. [79] sBCC were found to take place on sun-protected zones. nBCC and sBCC might be distinct tumour subtypes with different etiological factors involved: nBCC and iBCC might be due to chronic, long-term sun exposure, while sBCC might be due to the intermittent but intense sun exposure. [80,81]

BCC is a malignancy that increases in size with the passing of time. [82] Smaller tumors were found in the younger population, while the largest ones were found in the elderly patients. This particularity might be due to negligence (elderly people, especially those without relatives to help them, tend to address medical services less) or to the more careful attention paid by young people to their external aspect, but it might also be due to the presence of a more aggressive subtype of BCC in that age population (bsqBCC was found more frequently in the elderly, while sBCC was found in the young population).

Of particular interest is the observation that the largest tumor dimension and the largest tumor nest dimension are correlated and directly proportional, meaning that the larger the nests of malignant cells, the larger the tumors are, and inversely. Baker et al. (2021) state that tumor diameter is correlated to the metastatic risk, tumors measuring at least 4 cm having higher risks for spreading. [83] This allows the birth of the hypothesis that larger tumor nests could be correlated to increased risk for metastasis, but further research needs to be done in this particular direction.

The finding that Clark level III has the largest tumor nests might be explained by the fact that nBCC was the dominant type at this level. Vornicescu et al. (2021) sets the idea that a Clark level larger than III (and larger tumor sizes) might be a risk for tumor recurrence. [84] Whether larger tumor nests (implied by larger tumor sizes) indicate an increased recurrence risk remains to be seen.

The data presented in regards to tumor thickness reflect the aggressive, high-risk behavior of some BCC subtypes, increased tumor thickness being found mostly in iBCCs, bsqBCC and mBCC. This affirmation is supported by the findings of Gülseren et al. (2022), underlining the positive correlation between aggressive subtypes and tumor thickness. [85] Increased tumor dimension and increased tumor thickness can both be considered high-risk tumor predictors, having larger tumor nests and low lymphocytic reaction.

Host response to tumor development can be translated by the body's immune reponse to the neoplastic process through the lymphocytic reaction. Kaur et al. (2006) found

inflammatory patterns which correlated with tumor progression, a diminished host response being correlated with tumor extension. High risk BCCs were found to have less inflammation than low-risk ones. [86]

A suggestive sign of perineural invasion is considered to be chronic inflammation, [87-88] possibly predicting with higher accuracy the possibility of the presence of a more aggressive tumor or the recurrence risk. Perineural invasion and the suggestive signs thereof, seem to indicate more aggressive, high-risk tumors. Their presence was higher in elderly patients, those with larger tumors, in high-risk tumors – bsqBCC, iBCC, mBCC, mfBCC, in tumors with Clark levels IV and V (frequencies increasing steadily from Clark III), in high risk tumors (with or without a low risk component). Santos et al. (2017) support the findings of more frequent perineural invasion in larger tumors with more aggressive behavior. [89] Our findings in regards to ratio of positive perineural invasion cases surpass the ones found in current medical literature 20,1% versus 10% (reported by Hill et al. (2022)). [90] The fact that perineural chronic inflammation (suggestive signs for perineural invasion) was found parallel to perineural invasion suggests that this is a rather strong indicator for the presence of perineural invasion and, consequently, for incomplete excision, increased recurrence, making it necessary to highlight in the pathology report.

Angiogenesis, is a marker indicating tumor development and progression; the morphology and architecture of blood vessels can divulge information regarding the tumor's pathogenesis and diagnosis (especially with non-invasive techniques such as dermoscopy). Inflammation and hypoxia are drivers for (aberrant) angiogenesis which is an essential process for tumor development. [91] A study done by Bedlow et al. (1999) has found that the diameters of blood vessels in BCC cases are larger than those found in the normal skin, highlighting the fact that BCCs possess distinct tumor vasculature. [92]

The current study reported that larger vessel diameters were found in the elderly, in aggressive BCCs (bsqBCC, iBCC, mBCC, mfBCC) and in tumors having higher Clark levels (III to V, as opposed to those having a Clark level of II); the vessel diameters were found to be directly proportional to the tumor's and tumor nest dimensions (the larger the blood microvessel, the larger the tumor nests and the tumor itself).

9.3. Basal cell carcinoma's tumor-stroma cleft phenomena study. Known as "clefting", "the cleft" or even "the clefting artifact", this is a characteristic tumor trait which can be found in BCC, representing a separation found in-between neoplastic tumor nests and the stroma that surrounds them, up until recently considered to be a pathology laboratory

processing artifact, but with the help of innovative in-vivo investigative techniques, such as OCT and RCM it was found as a specific "structure" of BCCs, found in the skin of the patient, preceding the excision and processing steps. [29,93,94]

The current research has revealed that the BCC nests were found to always have an approximately equal dimension to the cleft, but were always larger than the cleft itself, being 1,04 times to even 317,77 times larger. A question arises whether the fact that nests being larger than the cleft itself could mean that the cleft's development might in fact be due to a mechanical factor; as the nests enlarge, apoptosis develops and although the peripheral palisading cells were connected to the basal lamina of the nest, pulling forces appear (from the center of the nodule to the periphery) putting strain on the hemidesmosomes of the palisading basal cells, which in turn give in to the mechanical strain.

Larger clefts, larger cleft-corresponding tumor nests and increased ratios for cleft-corresponding tumor nests and cleft itself, were found in nBCCs (considered low-risk); higher values were found in high-risk tumors. By these findings, the cleft itself might indicate a less aggressive course when it is larger (with smaller ratios between the nest and the cleft itself). The largest tumor nests' cleft width parallels the values found in the case of the largest cleft width's found in the tumor, with similar BCC subtype dependency, supporting furthermore our findings. Most BCC tumors had the cleft as a general characteristic, involving all tumor components – 89,3% and in all nests – ½ of cases. Clefting was found in all cases of sBCC, in over ½ of cases of smBCC and nBCC, and only in ¼ of mBCC, giving rise to a possible conclusion that the presence of the cleft (in all tumor components and all nests) might indicate a less aggressive behavior and possibly subtype.

When clefting was found in all tumor nests, every measurement was double the size of those found in the other cases (where celfting was not a general aspect characterizing all tumor nests and components): the largest cleft had twice the size, the largest cleft's corresponding tumor nest was twice as large, the largest tumor nests measured also doubled in size, along with its cleft dimension; this comes in support of the abovementioned statement of clefting being an indicator for a less aggressive behavior.

Clefts were characterized by either mucin (bluish-tinted material) or were optically clear, which might be a consequence of dislodging the material, as it has no support on which to fix itself on. The current study reveals that when clefting was found in all tumor nests, it was optically clear, and in cases of optically clear clefts, their maximum width was twice the size of clefts found in other cases, supporting the theory of dislodging.

The maximum width of the cleft was found in the current study to be directly

proportional to the tumor's thickness, an important parameter associated with tumor behaviour. [95,96] Also, the cleft was found to have its largest dimensions in low risk BCCs (with or without a high risk component), being almost doubled in size in such cases, with similar values for its corresponding tumor nest.

Similar larger clefts and larger corresponding tumor nests were identified in cases with a poor lymphocytic reaction (the lymphocytic reaction being a sign of the host's immune response to the tumor). [97] In the setting of immune suppression, could the cleft play a role in inhibiting the tumor's invasiveness and thus progression, being a sign of aggressive behavior inhibition (with aid from cell tumor apoptosis)?

Larger clefts and larger corresponding tumor nests were found to be directly proportional to the tumor's largest vessel diameter. As it would be expected, a better vascularized tumor ensures its larger growth, but why larger clefts? Large tumors imply high risk of recurrence. [98] Does the separation nest-stroma imply a lower risk of recurrence?

The current study has yielded other findings that have not reached the threshold of statistical significance: (1) optically clear material was associated with nBCC, smBCC, iBCC, with more superficial Clark levels, and squamous differentiation; (2) mucin/bluish material was associated with nBCC, iBCC, mBCC and mfBCC; it was also corelated with larger tumor dimensions, lerger tumor nests, increased tumor thickness, deeper Clark levels and follicular differentiation; (3) larger clefts and larger nests were associated with follicular differentiation; (4) larger clefts were found with squamous differentiation-associated tumors and with solar elastosis; (5) the cleft's maximum width and clefts found with the largest nests are larger in tumors associating mBCC, and deeper Clark levels; (6) smaller clefts were associated with perineural invasion or suggestive signs of perineural invasion. What might be drawn from the abovementioned statements is that smaller clefts were associated with a more aggressive behavior (supporting another finding in the current study), while larger clefts are associated with differentiating tumors towards follicular and squamous epithelia. Optically clear spaces were found to be linked to less aggressive subtypes.

CHAPTER 10 – Conclusions

10.1. General conclusions

1. The Severe Acute Respiratory Syndrome COronaVirus 2 (SARS-COV-2) infection, which was responsible for the Coronavirus disease 2019 (COVID-19) pandemic lowered the number of patients who sought medical care due to patient-dependent reasons and government limitations.

2. BCC patients often associate multiple pathologies, a repercussion of the routine implication of the elderly in such skin diseases, with heavy consequences on the optimal treatment management choice to be made.

3. The patients' urban environment of origin reflects the modern shift in BCC patients as past patients came from the rural areas.

4. BCC is frequently associated with other skin diseases, be it either inflammatory or neoplastic (benign or malignant).

5. Male BCC patients are more likely at risk for developing another NMSC, SCC.

6. Male patients are more likely at risk of developing BCCs, with increased likelyhood of them being high-risk tumors (such as bsqBCC and iBCC).

7. Body sites that are difficult to protect against UV radiation such as the forehead and nasal pyramid are preferential locations for BCC development.

8. Ulceration and solar elastosis were more frequently found in high risk tumors, but also nBCC and smBCC.

9. Aggressive, high-risk tumors (bsqBCC, iBCC, mBCC mfBCC) were found to be located more frequently on the sun-exposed areas of the human body: the forehead and the nasal pyramid.

10.2. Original contributions

1. The gender differences reported in our study are in contrast to the literature data, with younger BCC patients being male, while elderly subjects are female.

2. There is an inverse relation regarding allergy status and BCC development, most patients suffering from no allergies.

3. nBCC, iBCC and smBCC patients registered the highest number of prior BCCs – such aggressive behavior raising the possibility of including nBCC and smBCC in the high risk tumor group.

4. nBCC and iBCC are the most frequent BCC subtypes most likely to be associated with subsequent BCC development.

5. Certain BCC subtypes pose the risk of developing the same subtype in the future: iBCC is a risk for developing iBCC, similar for bsqBCC, while the nBCC tends to be a risk for developing nBCC or sBCC.

6. Smaller, higher-risk tumors (iBCC) are found more frequently on the glabrous skin, while non-glabrous skin registered larger tumors, but low-risk tumors (smBCC).

7. BCCs tend to be larger depending on several factors – patient's age (the elderly),

tumor location (forearm, thigh, anterior thorax – locations which allow a larger development/growth with little disturbance) and high-risk tumors (bsqBCC, iBCC).

8. Overall tumor dimension and tumor nest dimension are directly proportional; the larger the nests of malignant cells are, the larger the overall tumors.

9. Increased tumor dimension and increased tumor thickness can both be considered high-risk tumor predictors, having larger tumor nests and low lymphocytic reaction.

10. Follicular differentiation is a marker of low-risk tumors.

11. BCCs with squamous differentiation might be high risk and exert an aggressive behavior.

12. Suggestive signs of perineural invasion was mostly found in elderly patients, larger, high risk tumors – bsqBCC, iBCC, mBCC, mfBCC, with Clark levels IV and V, a possible marker for more aggressive, high-risk tumors.

13. Larger vessel diameters were found in the elderly, aggressive BCCs (bsqBCC, iBCC, mBCC, mfBCC) and in tumors having higher Clark levels (III to V); the vessel diameters were directly proportional to the tumor's and tumor nest dimensions, correlating with an aggressive behavior.

14. BCC nests have a minimally approximate equal dimension to the cleft, but are always larger than the cleft itself.

15. The cleft's maximum width and its corresponding tumor nest's width are dependent on the BCC subtype, large clefts being found in tumors such as: bsqBCC, iBCC, mBCC, mfBCC, but also in nBCC.

16. High-risk tumors tend to have smaller clefts, with higher ratios between the tumor nests and their corresponding cleft (even when the nests are small); the cleft itself might indicate a less aggressive course for the tumor when it is larger.

17. Larger clefts are optically clear, possibly due to a lack of support for mucin at this level, this material being dislodged during processing.

18. Larger clefts are directly proportional to the tumor's thickness and blood vessel diameter, the cleft possibly being a protective trait against further invasion.

19. Larger clefts are associated with tumors having a deeper pattern of growth and diminished host response, subsequently supporting their possible protective role from a possible further and deeper invasion.

10.3. Perspectives

1. As larger tumors have a higher risk of metastasis and larger tumors have larger

tumor nests, the latter could be correlated to an increased risk for metastasis.

2. Larger tumor nests (implied by larger tumor sizes) could indicate an increased recurrence risk.

3. nBCC might shift towards a high-risk tumor, when squamous differentiation is present.

4. Allergic patients might register poorer responses to tumor development, without having the same capacity for tumor inhibition as non-allergic patients.

5. As larger blood vessel diameters were found in more aggressive tumors, the possibility of angiogenesis inhibiting treatment arises.

6. Tumor nests being larger than their cleft could mean that the cleft's development might be due to a mechanical factor (pulling/tensile forces) due to cell nest apoptosis.

7. In the setting of a diminished host response to the tumor development, could the cleft play a role in inhibiting the tumor's invasiveness and thus progression, being a sign of aggressive behavior inhibition?

8. Clefting might represent a lower recurrence risk prognosis factor.

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