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WHAT WE NEED TO LEARN WHEN EXPLORING THE MIXED BASAL CELL CARCINOMA OF HEAD AND NECK

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Coexistence of different histopathological types of basal cell carcinomas (BCC) in the same anatomical localisation is rare, and, therefore, is engaging for histopathologists and clinicians. In many cases, the determination of a neoplasm type remains difficult, since BCC may consist of more than one histopathological subtype. Mixed BCCs often present with an aggressive course and recurrence when compared to other subtypes of a tumour. Furthermore, tumours of this type are associated with time-consuming treatment and not a very satisfactory cosmetic result, thus worsening the quality of the patient's life. Several clinical studies have been published regarding the histopathologically diverse tumours developed in the same anatomical region; however, largely peculiarities of mixed BCCs are not explored sufficiently. The purpose of this study was to substantiate the use of dermoscopy and morphology, assessing mixed type BCC of the head and neck. The tumours were removed with a surgical excision of 1 cm margins, and the tumour sites were assessed in a 24-month-long follow-up period. The dermoscopic characteristics of mixed and aggressive BCC are analysed in this study. Finally, to better estimate the invading cone of the tumour, a complex morphology, which included collagen type IV and podoplanin immunohistochemistry, and electron microscopy were used.

Key words: *mixed type, dermoscopy, podoplanin, collagen IV immunohistochemistry, electron microscopy.*

INTRODUCTION

Basal cell carcinoma (BCC) is a common malignant cutaneous tumour constituting up to 80% of registered non-melanocytic neoplasms (Abbas and Kalia, 2016; Muzic *et al*., 2017; Nolan *et al*., 2020). In European populations, the BCC tumour predominantly affects individuals with the skin phototype I and II, who have a 30% lifelong risk of developing BCC (Muzic *et al*., 2017). Basal and squamous cell carcinomas are the most prevalent skin malignancies in Latvia. The annual incidence of these types of tumours in the population of Latvia exceeds 1000 new registered cases. According to the data of the Centre for Disease Prevention and Control of Latvia, 448 new cases of skin cancer were detected in 2017, while the number of diagnoses in patients under the age of 35 years increased by almost 35% in two years (2016 and 2017) when compared to 2014 and 2015. In up to 80% of cases, BCC develops as a skin tumour of the head and neck (Goh *et al*, 2006; Ghafouri-Fard *et al*., 2010; Muzic *et al*., 2017). According to international estimates, the mortality associated with BCC is rather low (Abbas and Kalia, 2016; Muzic *et al*., 2017). Slow progression and rare distant spread simultaneously with often locally invasive and destructive growth are characteristics of the tumour first described by Jacob in 1827 (Mackiewicz-Wysocka *et al*., 2015). An increase in the number of patients who develop multiple BCC was demonstrated in recent years (Khalesi *et al*., 2013). This increase may be associated either with the

destruction of the ozone layer and longer exposure to ultraviolet radiation, or the spread of cancer (Laikova *et al*., 2019). Multiple tumours develop as multiple lesions in more than one anatomical localisation or demonstrate the coexistence of identical or different morphological types in the same or very close localisation (Bartoš, 2019).

Clinical manifestations of BCC vary; its differential diagnosis includes skin pathologies from a nevus and cutaneous squamous cell carcinoma to melanoma. Although a histopathological examination remains a standard diagnostic procedure, the use of advanced diagnostic tools, including dermoscopy, is encouraged (Verduzco-Martinez *et al*., 2013). Dermoscopy becomes essential when choosing a treatment method and assessing the characteristics of the tumour such as localisation, size, histopathological subtype, presence of pigmentation and residual lesions, as well as the risk of recurrence (Lallas *et al*., 2013; Popadič, 2014; Emiroglu *et al*., 2015; El-Sayed *et al*., 2020).

In most cases, BCC can be successfully treated; however, some patients are at high risk of tumour recurrence when lesions progress or become destructive. The recognition of the histopathological type of the tumour is mandatory for selecting an appropriate method for treating the tumour, in which more aggressive types require more argumentative treatment (Cohen *et al*., 2005). Commonly invasive growth of the tumour is associated with a high recurrence rate**;** therefore**,** a complex assessment of clinical, instrumental and pathological findings is needed when suggesting aggressive behaviour and a high risk of recurrence (Dandurand *et al*., 2006).

The common morphological forms of BCC, as well as the subtypes of these forms, are superficial, nodular, infiltrative, pigmented, and mixed (Madan *et al*., 2016). Among them, infiltrative, micronodular, mixed and metatypical BCC with admixed foci indistinguishable from squamous cell carcinoma are considered to be high-risk histopathological types (Marzuka *et al*., 2015). Mixed BCC is a subtype of BCC with mixed histology. A tumour of this type is a carcinoma that consists of two or more tumours in the same lesion. The diagnosis of mixed BCC is established histopathologically (Ghanadan *et al*., 2014). Mixed-type BCC, in contrast to other tumour types, is often manifested as a nodular type, and, therefore, is not recognised as an aggressive tumour by clinicians. Collagens are the most abundant protein polymers, which affect tumour tissue stiffness, regulate tumour immunity and contribute to its aggressiveness. Type IV collagen, as a major component of the basement membrane (BM), ensures its integrity and prevents the penetration of tumours deeper into the stroma (Khlebnikova *et al*., 2020). The destruction of the BM is associated with changes in the expression of type IV collagen, leading to an increase in the invasiveness of tumour cells (Tanjore *et al*., 2006). A single violation of the BM integrity associated with the expression of type IV collagen is not enough for the development of invasive cancer. Podoplanin controlling tumour cell motility and migration is a potential actor exhibiting a decisive effect for initiating tumour invasiveness and metastasis (Neinaa *et al*., 2020).

Underestimating the tumour, many choose a therapy that is suitable for the treatment of the non-aggressive types of BCC, which is absolutely not suitable for the aggressive forms of this tumour and ultimately leads to its recurrence. Therefore, assessing the biological behaviour of BCC, especially of the tumours with mixed structural appearance, the presence of more aggressive histological type should be suspected, leading to a proper selection of treatment tactics (Bartoš and Kullová, 2016). Despite the wide range of treatment modalities for BCC, the overall rate of recurrence is about 4–5% over five years (Wadhera *et al*., 2006; Kyrgidis *et al*., 2010). Highly invasive types of BCC commonly present with a higher incidence of recurrence. According to Sexton *et al*., the recurrence rate for the removal of superficial and nodular BCC varies from 3.6 up to 6.4%, whereas for micronodular it is 18.6% (Sexton *et al*., 1974). Surgery and radiation therapy become the methods of choice for most patients with high-risk BCC lesions (Telfer *et al*., 2012).

Due to the presence of certain dermoscopic and morphological correlations, some histopathological variants of BCC may be distinguished already during dermoscopic examination (Lallas *et al*., 2015). Aggressive forms of BCC, including scleroderma-like and infiltrative, demonstrate the presence of scattered arborising vessels in a whitish structureless region without clear boundaries (Longo *et al*., 2014). In contrast, mixed types of BCC remain poorly diagnosed during a dermoscopic performance and become a challenge to specialists. Dermatologists, pathologists and surgeons study these types of tumours to find out clinical features that could help in early diagnosis and choosing the right tactics to reduce the incidence of recurrence and advanced neglected tumours.

This study aimed to substantiate the use of dermoscopy and morphology, assessing mixed BCC of the head and neck.

MATERIALS AND METHODS

Twenty-nine patients clinically presented with suspected aggressive BCC of the head and neck, and treated prospectively in Rīga Stradiņš University, Institute of Stomatology, Department of Maxillofacial Surgery and the Oncology Centre of Latvia, within the timespan from 1 September 2016 to 1 September 2019, were enrolled in this study. In all twenty-nine cases, mixed type BCC was confirmed. All patients (18 male and 11 female patients) were examined clinically, dermoscopically, whereas the tissue samples — histopathologically. The age range was 37–90 years. The clinical data obtained from the patients were concerned with the duration and type of the lesion at the time of presentation, clinical and dermoscopic features, anatomical localisation and the size of the tumour. The disease relapse was monitored over a two-year follow-up period. The study was approved by the Ethical Committee of Rīga Stradiņš Uni-

versity, and written informed consent was obtained from all patients. The tumour tissue samples were taken following the tenets of the Declaration of Helsinki.

The dermoscopic diagnostic criteria for BCC included the presence of arborising vessels, short fine telangiectasia, leaf-like areas, large blue-grey ovoid nests, white streaks, ulceration, multiple small erosions, shiny white areas, in-focus dots, milky-pink to red areas, spoke-wheel areas, multiple blue-grey dots and globules (Puig *et al*., 2012; Lallas *et al*., 2013; Popadič, 2014; Wozniak-Rito *et al*., 2018).

The dermoscopic examination was conducted with a hand-held dermatoscope (3Gen DermLite DL3N with Pigment-boost; Olympus, USA) with a 30 mm ×10 lens. A polarised mode with both contact and non-contact techniques was used to visualise the dermoscopic findings of BCC in each lesion. A digital photography of clinical and dermoscopic presentation of the lesion was performed using a Samsung Galaxy S9+ (Samsung Electronics, Korea, Seoul) mobile camera. A semiquantitative assessment of dermoscopic findings was used (Argenziano and Zalaudek, 2007; De Vita *et al*., 2012; Okuboyejo *et al*., 2018). The levels appearing during dermoscopy were graded as follows: low – ≤ 25%, moderate – 26-70% and strong visualisation – > 70%.

The formalin-fixed, paraffin-embedded excised tumour tissues were processed and sectioned conventionally. The sections were mounted on SuperFrost Plus Adhesion slides (Gerhard Menzel GmbH, Germany), whereas parallel sections — mounted and routinely stained to diagnose the type of BCC. The histopathology of the tumour was assessed by two independent observers following the classifications of World Health Organisation (WHO). Based on the last WHO classification of skin tumours revised in 2018 and recent publication (Elder *et al*., 2018), the presence of either low-risk BCC presented by nodular, superficial, pigmented, infundibulocystic and fibroepithelial, or higher-risk BCC presented by basosquamous carcinoma, sclerosing/morphoeic, infiltrative, micronodular and BCC with sarcomatoid differentiation was confirmed.

For immunohistochemical reactions, the sections were incubated overnight at 4 °C with the following primary mouse monoclonal antibodies: anti-collagen IV type (Dako Denmark A/S, Glostrup, Denmark, clone CIV 22, 1 : 25), which labels the *lamina densa* of the basement membrane, and anti-podoplanin (Abcam, Cambridge, MA, USA, clone PDPN/1433, 1 : 200), which mediates a pathway leading to cell migration and invasion *in vivo* and *in vitro* (Wicki *et al*., 2006).

The amplification of primary antibody and visualisation of reaction products were performed applying the HiDef Detection HRP Polymer system (CellMarque, Rocklin, CA, USA); after rinsing in phosphate-buffered saline (PBS) solution, sections were incubated with HiDef Detection™ Amplifier for 10 min at room temperature (RT) and HiDef Detection™ HRP Polymer Detector for 10 min (RT), respectively. The antigen sites were visualised with 3,30 diaminobenzidine (DAB) tetrahydrochloride kit (DAB+ Chromogen and DAB+Substrate buffer, Cell Marque, Rocklin, CA, USA) applied for 5 minutes. The cell nuclei were counterstained with Mayer's hematoxylin, washed, dehydrated, cleared, mounted in Roti® Histokitt (Carl Roth, Karlsruhe, Germany) and cover-slipped. The sections were further analysed, and the results of immunohistochemical reactions were evaluated semiquantitatively using the gradation system proposed by Marasą *et al*. (2008). The assessment of immunostaining was performed semiquantitatively in 20 randomly selected visual fields of each sample (magnification 400×) representing the regions of interest. Immunostaining for collagen type IV was confined to the BM and displayed a linear (continuous and discontinuous) pattern. In turn, podoplanin revealed cellular membranous expression, including coloration of the membranous portion of cell projections.

The sections were photographed using a Leica light microscope (LEICA, LEITZ DMRB, Wetzlar, Germany) with a DFC 450C digital camera and scanned by a Glissando Slide Scanner (Objective Imaging Ltd., Cambridge, UK).

For a better exploration of the ultrastructural peculiarities of tumour cells forming an invasive cone, a transmission electron microscopy (TEM) was used. The tissue samples were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated through graded ethanol series and embedded in epoxy resin (Sigma-Aldrich). The ultrathin sections of thickness 70–80 nm were cut with LBR ultramicrotome, collected on formvar-coated grids, double-stained with uranyl acetate and lead citrate and examined with a JEM 1011 electron microscope (JEOL, Japan). The specimens were examined at magnification ×8000–×50 000.

Statistical data analysis with the help of SPSS version 26.0 software was performed to assess the dermoscopy and immunohistochemistry results. The diagrams were acquired using the Amchart software. To test whether the collected numerical data were normally distributed, a Kolmogorov–Smirnov normality test was applied. The quantitative data were expressed as means ± standard deviation, whereas categorical parameters were expressed as frequencies and percentages. The Pearson's rank correlation coefficient was used to estimate the relationships between the immunostaining patterns of the antibodies used in this study. The correlation between antigen expression and histopathological type of BCC was studied by Chi-Square statistics. The Friedman Chi-squared test was used to estimate relationships between the dermoscopic pattern and a morphological type of the tumour. In the case of paired group comparisons, the Wilcoxon matched-pairs signed rank test with Bonferroni correction was used. p values of < 0.05 were considered significant.

RESULTS

Twenty-nine cases were diagnosed histopathologically as mixed type BCCs. One case was interpreted as a rare case

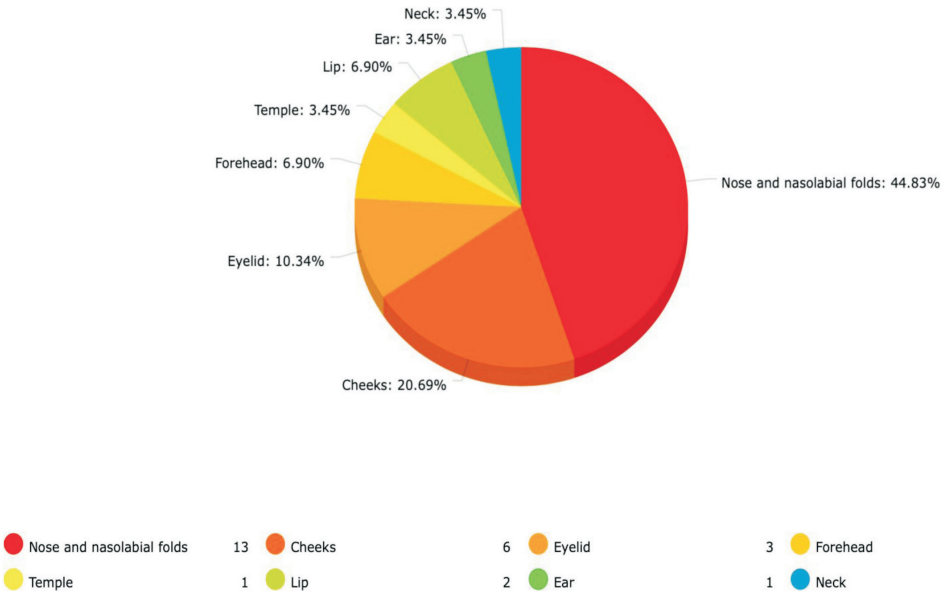

Fig. 1. Frequencies of the mixed type BCC demonstrated in different regions of the head and neck.

and included a combination of a solid-adenoid- and infiltrative arrangement of tumour cells in one tumour. The most frequent combinations of the mixed BCCs included nodular-infiltrative, nodular-superficial and nodular-micronodular types. Anatomically, the tumours were located as follows: one (3.45 %) on the temple, two (6.90%) on the forehead, 6 (20.69 %) on cheeks, 13 (44.83 %) on the nose and nasolabial folds, one (3.45%) on the ear, one (3.45%) on the neck, three (10.34 %) on the eyelid, and two (6.90 %) on the lip (Fig. 1). During the study, 18 tumours further developed as recurrent neoplasms. Among them, seven tumours developed after a complete surgical excision with clean histopathological margins, whereas 11 — after mini-invasive treatments such as cryotherapy, imiquimod therapy and laser ablation. Among 18 recurrent BCC tumours, ten neoplasms were diagnosed as a mixed type.

In most cases, the histopathologically aggressive mixed type BCC was localised on the nose and nasolabial fold, and commonly presented as nodular-infiltrative or solid-adenoid tumours. The size of mixed tumours was greatly varying from 0.2 mm to 2.5 cm, and, occasionally, up to 4 cm (Table 1).

Dermoscopically, 29 cases of aggressive BCC were suspected. The characteristic dermoscopic findings observed in aggressive BCC are presented in Figure 2A, B and summarised schematically in Figures 3 and 4. The most common vascular pattern of mixed BCC was the presence of arborising vessels and short-fine telangiectasias, mostly found in small-sized BCCs. Shiny white areas (16 patients, 55.1%), white streaks (19 patients, 65.4%), milky-pink to red areas (23 patients, 79.3%), ulceration (10 patients, 34.4%), and multiple small erosions (11 patients, 26.5%) appeared to be frequent dermoscopic findings. In turn, pigmented structures distinguished as blue-grey globules (three patients, 10.3%), blue-grey ovoid nests (four patients, 13.7%) and in-focus dots (two patients, 6.8%) were less frequently diagnosed. The pigment-associated lesions were commonly

Table 1. Distribution of mixed type BCCs by anatomical localization and size

Localisation	Number of cases	Min size	Max size	The average estimate
Nose	13	0.3 cm	4 cm	1 .1 cm
Cheek	6	0.2 cm	3.2 cm	1.85 cm
Temple	1	0.5cm	0.5 cm	0.5 cm
Forehead	2	0.3 cm	0.7 cm	0.5 cm
Eyelid	3	0.2 cm	0.6 cm	0.4 cm
Lip	2	0.3 cm	1.3 cm	0.8 cm
Ear	1	0.8 cm	0.8 cm	0.8 cm
Neck	1	2.8 cm	2.8 cm	2.8 cm

BCC, basal cell carcinoma; Min, minimal; Max, maximal

shaped as maple leaf-like areas (two patients, 6.9%) and spoke-wheel areas (one patient, 3.4%).

The frequency of dermoscopic findings recognised in mixed BCC is summarised in Table 2. The presence of arborising vessels was the most common dermoscopic finding in BCC. Importantly, arborising vessels were found to differ in all BCC types. In this study, the arborising vessels were most often diagnosed in mixed BCCs with a nodular component. In mixed superficial BCCs, arborising vessels were accompanying milky-pink to red areas, multiple erosions, and short fine telangiectasia. Blue-grey ovoid nests were the most common features of pigmented BCCs, whereas white streaks in infiltrative BCCs.

To evaluate the invasion pattern of mixed BCCs, collagen type IV and podoplanin immunohistochemistry was performed. The immunoexpression was greatly varying, demonstrating continuous, discontinuous, and lacking expression (Fig. 5A). When estimated, mixed BCCs presented with 49.47% tumours, revealing the absence of collagen type IV expression, 40% — revealing discontinuous decoration, and only 10.53% — demonstrating the continuous pattern of the BM. In highly infiltrative variants of BCC, colla-

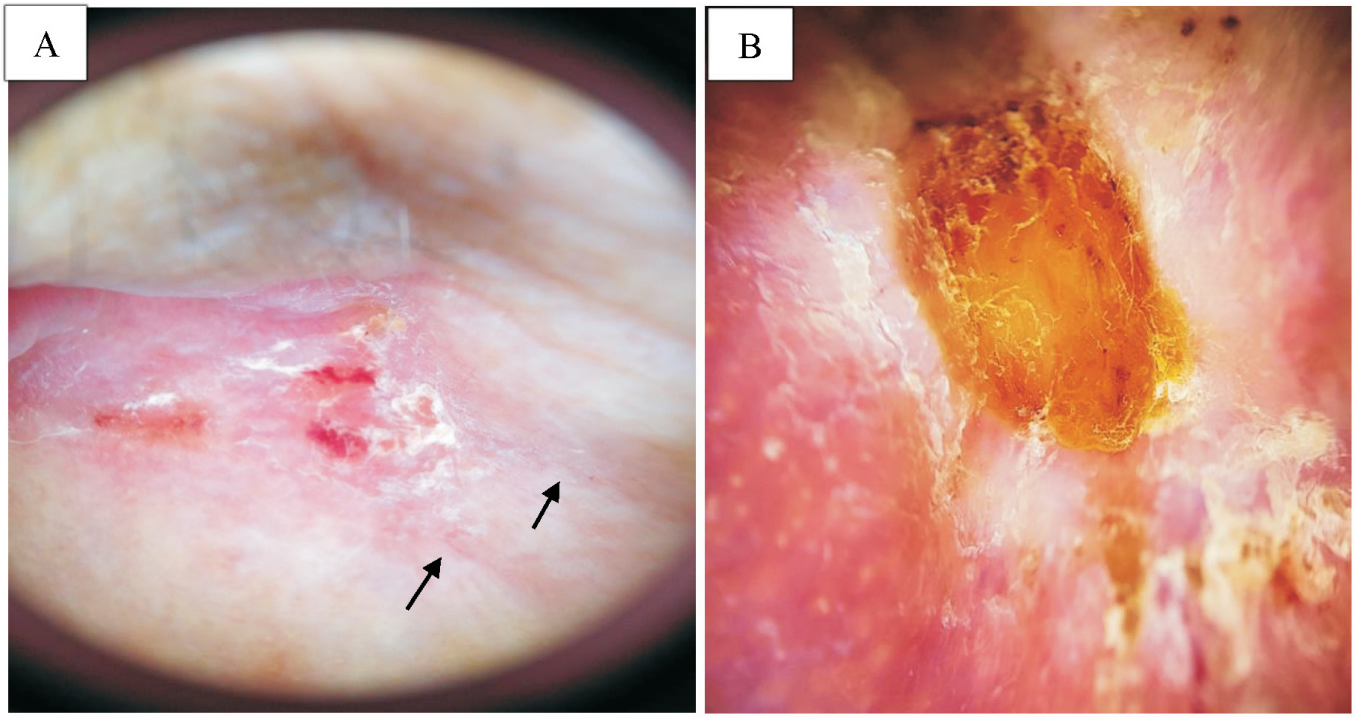

Fig. 2. (A) Homogeneous shiny structureless areas with the milky-pink background; superficial short-fine telangiectasia and few fine arborising vessels. The tumour surface with multiple small erosions, and dermoscopically not clearly detectable edges in the case of primary mixed type BCC, which histopathologically was recognised as superficial and infiltrative type. Filamentous and thread-like slender cords beyond the visual boundaries of the tumour characteristic of more aggressive BCC demonstrating a high risk of recurrence (black arrows). (B) A homogeneous white-to-pink background and ulceration in the middle of the tumour demonstrated dermoscopically in the case of recurrent mixed type BCC, which histopathologically was recognised as nodular with cysts and infiltrative type.

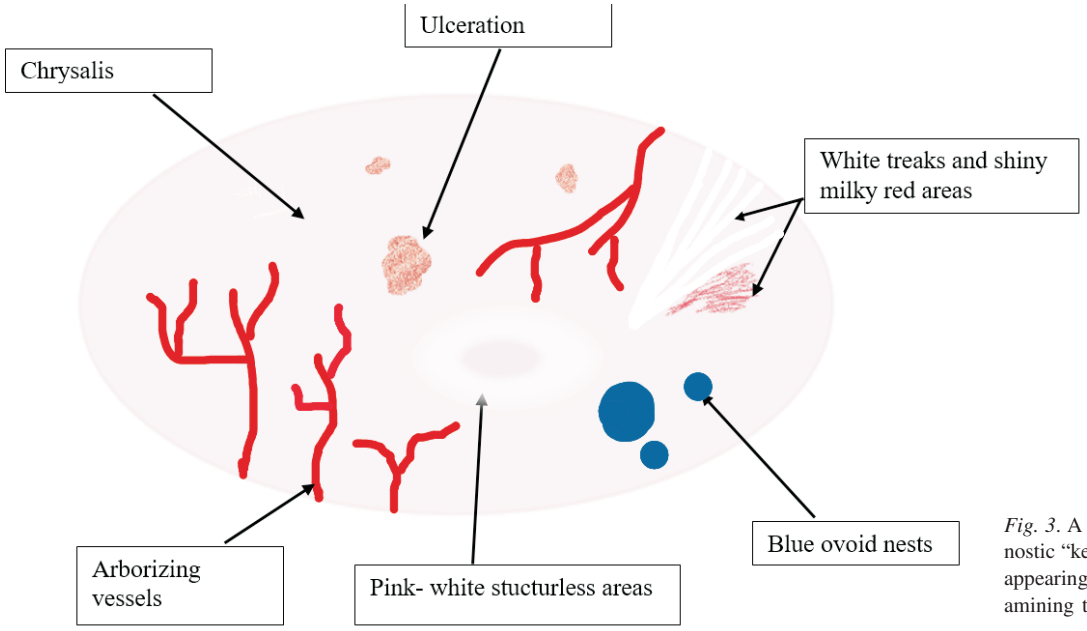

Fig. 3. A scheme, which depicts diagnostic "keys" for the mixed BCC type appearing dermoscopically when examining this type of tumour.

gen expression was absent in up to 96% of tumours. Collagen type immunostaining was recognised as a linear decoration along the basal aspect of the tumour cord or nest (Fig. 5B, C).

Among 29 mixed BCC samples were analysed, 58.6% expressed podoplanin. Some tumours did not express podoplanin (Fig. 6B, C, D), whereas others expressed podoplanin exclusively in the invading front (Fig. 6A, B). Finally, some tumours expressed podoplanin within the basal cell layer with frequent cytoplasmic staining (Fig. 6A, C, D).

Finally, for better assesment of tumour architecture in the front, the cellular morphology was explored using TEM. The cells displayed an irregular cell shape, and the intercel-

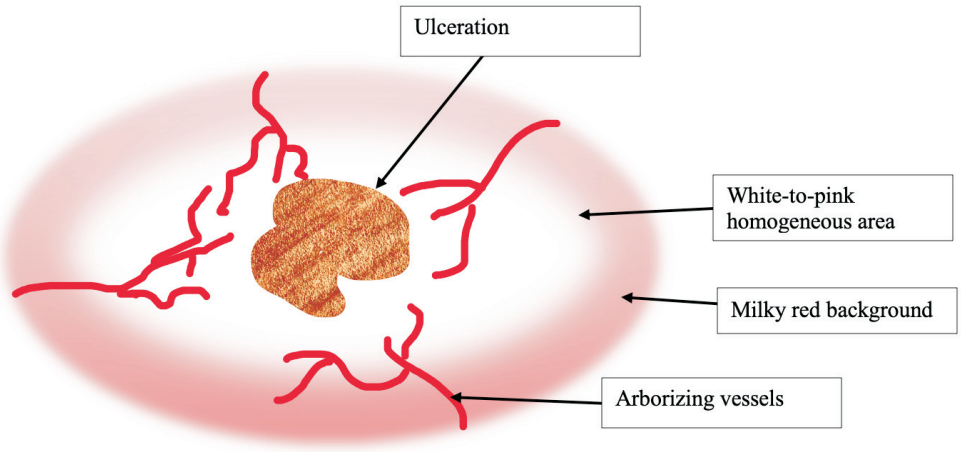

Fig. 4. A scheme, which depicts diagnostic «keys» for the infiltrative sclerosing BCC type appearing dermoscopically.

Table 2. The prevalence of dermoscopic findings in mixed BCC

Dermoscopic findings	No visualization, %	Visualization < 25%	Visualisation > 25–70%	Visualisation > 70%
Arborizing vessels	9 (31.0)	8 (27.6)	4 (13.8)	8 (27.6)
Blue-grey ovoid nests	25 (86.2)	0	1 (3.4)	3 (10.3)
Concentric structures	23 (79.3)	0	3 (10.3)	3 (10.3)
In-focus dots	27 (93.1)	1 (3.4)	0	1 (3.4)
Maple leaf-like areas	27 (93.1)	0	0	2 (6.9)
Milky-pink to red areas	6 (20.7)	4 (13.8)	2 (6.9)	17 (58.6)
Multiple blue-grey dots	26 (89.7)	0	2 (6.9)	1 (3.4)
Multiple small erosions	21 (72.4)	1 (3.4)	3 (10.3)	4 (13.8)
Shiny white areas	13 (44.8)	1 (3.4)	6 (20.7)	9 (31.0)
Spoke-wheel areas	28 (96.6)	0	0	1 (3.4)
Short fine telangiectasias	10 (34.5)	2 (6.9)	7 (24.1)	10 (34.5)
Ulceration	19 (65.5)	2 (6.9)	3 (10.3)	5 (17.2)
White streaks	10 (34.5)	1 (3.4)	3 (10.3)	15 (51.7)

BCC, basal cell carcinoma

lular spaces were dilated (Fig. 7A, B). Almost all cellular cords presented with loss of cell-to-cell junctions; only primitive junctions were preserved. There were only occasional tonofilaments observed in the cytoplasm. The changes of the BM included the presence of multi lamination, splitting and development of a discontinuous course.

DISCUSSION

Despite the high incidence of BCC, there are very few studies describing the peculiarities of different subtypes of the tumour and likely differences between them. There are various methods of treating BCCs, ranging from minimally invasive methods to surgical therapy, in which the histopathological findings of the tumour is one of the most determining factors in choosing an appropriate treatment method (Drucker *et al*., 2018). The specialists involved in the diagnostics and treatment of BCC should be aware of the successful and safe management of patients due to frequent recurrence, even in the case of a complete primary surgical removal of the tumour (Paoli *et al*., 2019).

According to the results of the present study, the majority of mixed-type BCCs are localised on the face, nose and nasolabial area. These are sun-exposed skin regions of the face. Furthermore, most mixed-type BCCs have a larger size than the tumours of other subtypes. Most mixed-type BCCs have a common nodular component, which might have an association with the stem cells of the hair follicles (Peterson *et al*., 2015). By analysing dermoscopic and histopathological correlates, clear evidence comes to light — the aggressive potential of tumour does not increase only by enlargement of neoplasm (Roldán-Marín *et al*., 2014; Emiroglu *et al*., 2015; Enache *et al*., 2019).

These findings are supported also by other authors; localisation of aggressive tumours should be taken into account, since these are often localised in the areas traversed by large-sized arteries (Lammers *et al*., 2011; Karaninder *et al*., 2012). Routinely, the diagnosis of BCC is established by histopathological examination after the removal of a suspicious mass. However, in recent years, diagnostics of skin tumours was improved by using a non-invasive and cheap *in vivo* dermoscopic examination method. This modern and convenient method of examination allows practicioners to study the morphological features of tumours that are not obviously visible, thus contributing to the accuracy of the diagnosis.

The most frequent dermoscopic finding of mixed BCC is the presence of arborising vessels and short-fine telangiectasias. Shiny white areas, white streaks, milky-pink to the red background, ulceration and multiple small erosions were also observed more often than the other findings. The afore-

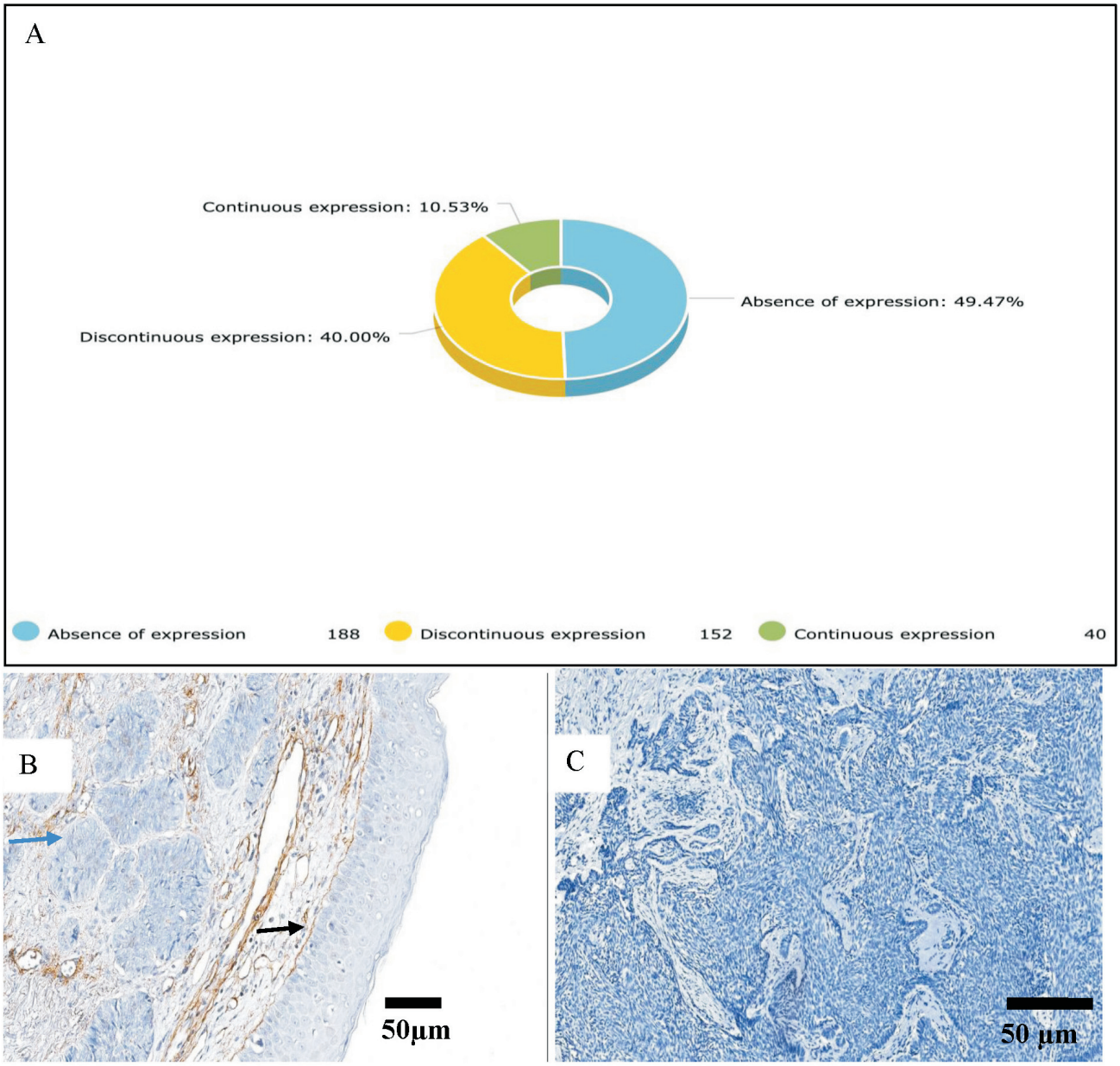

Fig. 5. Collagen type IV expression in the mixed type BCC. Panel (A) shows frequencies of the absence of collagen expression and the presence of continuous and discontinuous patterns confirmed immunohistochemically. Panels (B and C): collagen type IV immunohistochemistry. The panel B demonstrates the presence of linear structures along the basal aspect of the epidermis (black arrow), which reflects the immunohistochemical appearance of collagen type IV within *the lamina densa* of the basement membrane displaying continuous pattern, which becomes discontinuous when enveloping small nodules of the tumour mass (blue arrow), ×200. Panel C demonstrates the tumor nodules displaying infiltrative growth and the absence of collagen type IV expression, ×100.

mentioned dermoscopic criteria recognised by the authors of this research as suitable findings when suggesting aggressive BCC are in accordance with the results of other authors (Emiroglu *et al*., 2015; Enache *et al.*, 2019). The appearance of arborising vessels, ulceration, white streaks, and milky pink to the red background is consistent with peritumoural inflammation often found in aggressive BCC (Zalaudek *et al*., 2010; El-Sayed *et al*., 2020). Dermoscopically, upon the detection of a lesion with a probability of neoplasmic transformation, a specialist should treat it as a tumour with an increased risk of recurrence.

In the present study examining the aggressiveness of mixed BCC by the use of morphological methods, a special interest was shown in exploring the invading cone of the tumour mass. An invasive growth is highly dependent on epithelial-mesenchymal interactions, when a tumour cell gradually loses its adhesive properties, detaches from the BM and continues migration into the underlying connective tissue (Rowe, 2008). During this process, the apical-basal polarity of the epithelial cell is lost, intercellular junctions become more primitive, the cytoskeleton rearranges, and matrix metalloproteinases are synthesised increasingly and degrade the main components of the BM, one of which is type IV collagen (Rowe, 2008; Khlebnikova, 2020).

The destruction of type IV collagen within *the lamina densa* of the BM is significant and crucial for the germination of

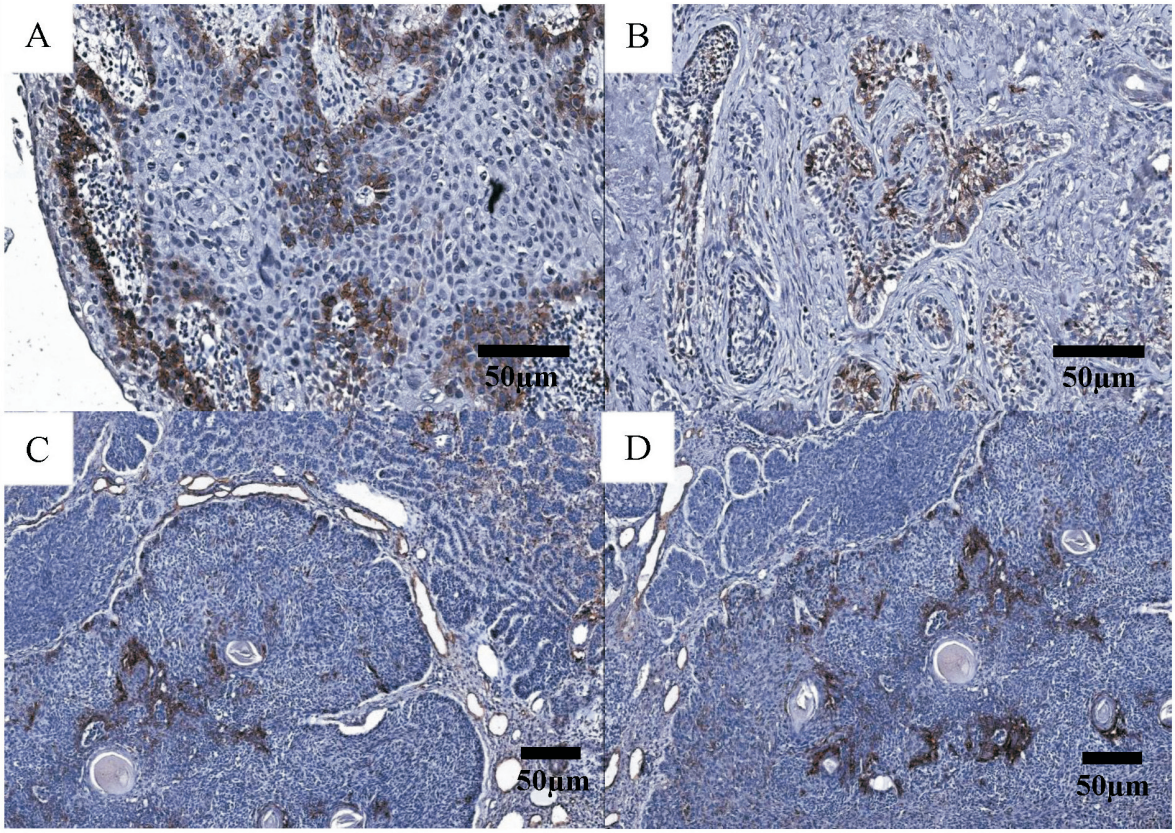

Fig. 6. Podoplanin immunohistochemistry. (A) The irregularly shaped nodular mass, demonstrating invasive growth into the stroma in primary BCC. Most of the cells at the basal aspect of the invading tumour express podoplanin, ×200. (B) Some tumour nests and strands of mixed recurrent BCC with infiltrative growth, demonstrating podoplanin immunopositivity, whereas some — the absence of immunostaining, ×200. (C, D) Mixed-type BCC presents with a large nodule demonstrating podoplanin expression along the basal aspect and within the tumour mass, and multiple tumoral nests and strands revealing micronodular architecture and lacking the expression, ×100.

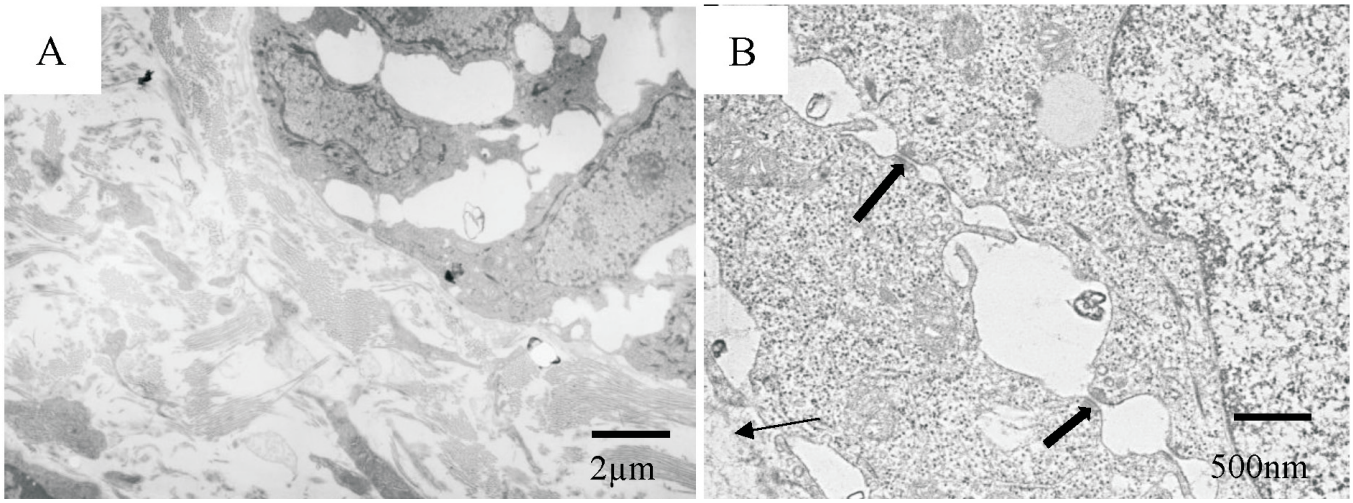

Fig. 7. The ultrastructural appearance of tumour cells forming an invasive cone in the case of mixed BCC on transmission electron microscopy. (A) Low-power view of the outer edge of the invading tumour surrounded by collagenous stroma, ×4 000. (B) Fragments of two tumour cells separated by dilated intercellular spaces; there are only primitive cellular contacts preserved (thick arrows); the basement membrane is recognised by the lamina densa displaying discontinuous appearance (thin arrows), ×15 000.

tumour cells and the infiltration of the underlying connective tissue (Fang *et al*., 2014). Changes in collagen type IV expression along with the formation of infiltrative growth patterns and tumour budding are demonstrated in aggressive bladder tumours (Miyake *et al*., 2017). It is believed that collagen changes cause biomechanical signals in a supportive scaffold, which, in turn, are sensed by both tumour cells and stromal cells, thus**,** triggering a cascade of biological events (Fang *et al*., 2014).

In this study, a violation of the integrity of the BM and the disappearance of collagen type IV collagen leading to an in-

crease in the invasive potential of the tumour in mixed and infiltrative BCC was demonstrated. This evidence is partly in agreement with studies that showed differences in the expression of type IV collagen, distinguished as linear and continuous in the superficial BCC and almost nil in the micronodular and infiltrative BCC (Chuprov, 2008; Arduino, 2010; Khlebnikova *et al*., 2020). The ability of mixed and infiltrative forms of BCC to destroy the basement membrane can be associated with their invasive potential and should be considered in the early diagnosis of neoplasm and prediction of the biological behavior of BCC (Crowson, 2006).

Apart from the assessment of collagen type IV impairment, the expression of the podoplanin marker was estimated and ultrastructural peculiarities of BCC at the invading cone was analysed. Studies exploring the expression of tumour-associated genes in squamous cell carcinoma have already suggested the role of podoplanin in normal and malignant homeostasis of the epidermis (Acton, 2012; Baars *et al*., 2015). Previous studies have highlighted the role of podoplanin in the induction of collective and single tumour cell migration (Wicki and Christofori, 2007). In this study, we demonstrated irregularly shaped invasive tumour cords highly decorated with the anti-podoplanin antibody along the basal aspect and reflecting aggressive neoplastic potential. Simultaneously, large and smoothly delineated nodular tumour masses demonstrated a different pattern of podoplanin expression and even its absence. These differences in podoplanin marker expression may somehow reflect the peculiarities of a tumour growth in BCC and are partly consistent with the results of other authors (Wojciechowska-Zdrojowy *et al*., 2016). Additionally, we observed an increase of podoplanin expression demonstrated at the invading cone and paralleled by a decrease of cytoplasmic tonofilaments, simplification of cellular junctions and the discontinuity of the BM assessed ultrastructurally.

In this case, a high frequency of the combination of nodular-infiltrative, nodular-superficial and nodular-micronodular types in mixed BCC was confirmed by the use of histopathology. Clinically, the nodular form of BCC remains one of the most commonly diagnosed, whereas dermoscopically, nodular, superficial and infiltrative BCCs are often distinguised by the presence of tumour-associated diagnostic criteria. Aggressive BCC demonstrates impairment of the integrity of the BM and higher expression of podoplanin at the invasive front. However, a more extensive analysis of the cellular and molecular mechanisms that govern the tumour invasion process warrants further investigation.

CONCLUSION

The coexistence of two or more different histopathological types of BCC in the same anatomical position is very rare. Nevertheless, one should suspect and explore such coexistence when faced with BCC. Such coexistence can include a large area and requires extensive surgical removal and grafting of the skin. BCC biopsy is recommended before choosing non-surgical treatment methods. These timely therapeutic procedures are mitigated in preventing relapses and metastases.

Histopathological examination showed that dermoscopy is 100% accurate when diagnosing BCC. Due to the developed dermoscopic algorithms, it is possible to suspect BCC with a tendency to an aggressive course. In this context, filamentary thin strands extending beyond visually recognised borders of the tumour may be suggestive of aggressive BCC. However, further studies should be conducted to understand whether these algorithms are sufficient for error-free forecasting of aggressive subtypes of BCC. The morphological assessment applied in this study proved the necessity of further exploration of the molecular players and mechanisms responsible for the better understanding of an invasion process in mixed BCC. Due to a rather small number of patients, this study has a certain limitation, and the results should be tested on larger cohorts.

The authors declare that there is no conflict of interest.

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KAS JĀŅEM VĒRĀ, PĒTOT GALVAS UN KAKLA RAJONA JAUKTA TIPA BAZĀLO ŠŪNU KARCINOMU

Dažādu bazālo šūnu karcinomas (BŠK) histopatoloģisko tipu pastāvēšana vienā anatomiskajā lokalizācijā ir reta. Šīs patoloģijas izpēte izraisa gan morfologu, gan klīnicistu interesi. Iepriekš veiktajos pētījumos demonstrēts, ka jaukta tipa BŠK bieži manifestējas ar agresīviem audzēja apakštipiem. Turklāt šie audzēji ir saistīti ar laikietilpīgu ārstēšanu, nepārliecinošu kosmētisko rezultātu, un tā rezultātā pacienta dzīves kvalitāte neuzlabojas. Literatūrā apskatīti vairāki klīniski gadījumi par diviem dažādas struktūras audzējiem, kas attīstījušies vienlaicīgi, bet par jaukta tipa BŠK viena audzēja ietvaros pētījumu dati ir ierobežoti. Šī pētījuma mērķis bija izvērtēt dažādas jaukta tipa BŠK, izmantojot dermoskopijas un morfoloģijas analīzes iespējas. Audzēju audi tika iegūti ar ķirurģisko ekscīziju ar 1 cm atkāpi veselu audu robežās, kam sekoja novērošana 24 mēnešu laikā pēc ārstēšanas. Pētījumā noskaidroti jaukto tipu BŠK dermatoskopiskie kritēriji, kas ir nozīmīgi pareizas ārstēšanas taktikas izvēlei. Kompleksi izmantojot morfoloģijas metodes, kas ietvēra IV tipa kolagēna un podoplanīna ekspresijas imūnhistoķīmisko analīzi un elektronu mikroskopiju, izpētīta audzēja invāzijas fronte.