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RESEARCH ARTICLE

Role of Bcl-2, Apoptotic Index, and Ki-67 Expression in Basal Cell Carcinoma and their Association with Aggressive and Non-Aggressive Histological Phenotypes

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ABSTRACT

Background
The purpose of this paper was to evaluate the association of the Bcl-2 protein, Ki-67 antigen expression and the apoptotic index with biological behaviour of basal cell carcinoma.

Methods
Combined analysis of retrospective and prospective study of data from two centres over a period of years 2008–2023 on histologically confirmed basal cell carcinoma without any age limitation. Selected prognostic markers related to the aggressive or non-aggressive tumour types were evaluated.

Results
The study cohort included 66 patients with 68 basal cell carcinomas of the eyelids (n = 68). The median age was 68 years. Resection margins < 2 mm were in 33.9 %, without any connection to recurrence rate (p = 0.076). Average value of Bcl-2 expression in non-aggressive types was 82.65 % (p = 0.000). The value of Ki-67 expression in patients with non-aggressive tumours amounted to 35.49 % (p = 0.068). Non-aggressive tumours most frequently exhibited the apoptotic index of Grade I (p = 0.535). No Grade III case was observed in the aggressive types. In two cases, the orbital exenteration was carried out (0.03 %). In one case was administered biological therapy. HDR brachytherapy was applied in 6 cases. A recurrence of the disease was observed in 4 cases (0.06 %).

Conclusion
New information on cancer biomarkers in basal cell carcinoma contribute to choosing a correct therapy with achieving good aesthetic results and a good survival rate.

Keywords: Bcl-2 protein, Ki-67 antigen, apoptotic index, basal cell carcinoma, onco-ophthalmology

Introduction

The incidence of non-melanoma skin carcinoma, including eyelid tumours, shows a rising trend. The most frequently occurring malignant eyelid carcinoma is the basal cell carcinoma (BCC).[1] Basal cell carcinomas are the most common tumours afflicting the Caucasian population. It is usually not fatal, but if it is not diagnosed for a long time, the function and the appearance of the eyelid will be destroyed. Basal cell carcinoma with orbital and perineural invasion is uncommon, with a reported incidence of only 1.6 %–2.5 %.[1] BCC of the eyelids has a high risk of recurrence, especially in infiltrative types. Based on the evidence obtained in multiple published studies, there is a provable correlation between the gender, the age, and the incidence of this disease.[2,3] The malignant tumour diagnosis may only be definitively confirmed based on histopathological examination of a collected tissue sample. In most cases, the primary treatment of eyelid carcinoma is surgical resection. Advanced lesions require extensive surgical interventions, as well as the application of other available therapeutic modalities. Over the last years, there has been an increasing interest in the development of biomarkers with the aim of improving the prevention and ensuring early detection of cancer with personalised therapy management.[4]

There are multiple risk factors that contribute to the development of new BCCs of the eyelids. Together with biomarkers, those risk factors determine the disease development, biological behaviour, as well as the subsequent management of the patient who eventually presents with this type of carcinoma. Risk factors include the age, the male gender, skin phototype 1 and 2, a frequent exposure to UV radiation and frequent sunburns, severe actinic damage, previous radiotherapy in the medical history, an increased number of identified BCCs, a tumour size above 1 cm, the width of the lesion margin, the family history with other types of skin cancer, a low DNA repair capacity, detected tumour necrosis factor (TNF), microsatellite polymorphism, polymorphism of the PTCH gene, as well as polymorphism of glutathione S-transferase and P450 cytochrome.[5] The identification of manageable risk factors facilitates the implementation of preventive actions and screening with the aim of reducing the constantly growing incidence.

Biomarkers are molecules which, when detected or assessed, provide information on a disease beyond the standard clinical parameters. Prognostic biomarkers facilitate the identification of high-risk tumours for the purpose of targeted screening and targeted primary and secondary prevention for early detection and treatment.[6] One of the mechanisms engaged in the tumour growth is that the cell escapes the cell death – apoptosis. In physiological conditions, apoptosis is controlled by the CD95 cell receptor. The mechanism of apoptosis is also facilitated by other proapoptotic genes (BAD, BAX, BID, and p53) and apoptotic inhibitors (Bcl-2, BCL-X). In cancerous cells, the role of apoptosis is to intervene through mutations in the controlling genes that regulate apoptosis in normal cells. Bcl-2 family proteins determine whether a particular cell dies or survives by controlling the mitochondrial apoptosis in the cell division process. Non-Bcl-2 family proteins emerge as new regulators of apoptosis and are currently subjected to pharmacological research. A cell proliferation degree correlates with a tumour growth rate. More proliferation is associated with a lower degree of differentiation, more aggressive biological behaviour, and a worse prognosis of the malignant disease.[7] The proliferation activity is most frequently identified based on immunohistochemical tests, in particular using the antibody against the Ki-67 antigen. The Ki-67 antigen is a non-histone protein.[8] The apoptotic index (AI) is regarded as an important, yet still unexplained, prognostic factor of biological behaviour of tumours. AI is calculated as the number of apoptotic cells and apoptotic bodies, expressed as the percentage of the total number of tumour cells counted in each case in a histology sample.[9]

Methods

This paper is based on the persisting need for understanding new prognostic parameters of basal cell carcinoma that determine a personalised approach to the individual patients. It is a combined analysis of retrospective and prospective study with patients presenting with malignant lesions in the eyelid region.

The cohort consisted of the patients treated in the UVEA Eye Clinic in the period from 2018 to 2023 and the patients treated in the Department of Ophthalmology of the Central Military Hospital in Ružomberok in the period from 2008 to 2021. In these periods the oculoplastic surgery was carried out. Cohort asymmetry had no impact on results. We did not examine the impact of the environment. Inclusion criteria comprised: histologically confirmed basal cell carcinoma in the eyelid region, no age limitation, no size of tumours limitation. Exclusion criteria encompassed: another histological type of skin eyelid lesion. Patients suspected to have a tumour were indicated for surgical resection including the histological examination of the excised sample. Those patients were monitored for the epidemiological parameters, such as the gender and age, the location of their malignant tumour and the application of any other therapy. The selected surgical approach depended on the tumour location and size and was performed following the recommended procedures. This paper presents the evaluation of the resection margin size and of the R0/R1/R2 resection types in terms of the risk of recurrence of malignant cancer.

One of the key objectives of this paper was the tumour evaluation from the histopathological and immunological point of view. Samples were divided into two groups based on the histological classification – aggressive (infiltrative, sclerosing, morpheaform, micronodular, infundibulocystic) and non-aggressive tumours (nodular, superficial, cystic). The subject of the evaluation included selected prognostic markers related to aggressive or non-aggressive tumours, as well as the recurrence and metastases. The Bcl-2 expression was calculated as the total percentage of positive tumour cells in the carcinoma. The value of the Ki-67 index was calculated as the average percentage of proliferating tumour cells in a carcinoma. The apoptotic index was evaluated through the semi-quantitative evaluation of apoptosis.

Regarding the determined hypotheses and the nature of the related data, the data was statistically analysed by applying the Chi-square test for data distribution, the Cramer's V test, the Phi Coefficient test, the Kolmogorov-Smirnov test for normality, the Shapiro-Wilk test for normality, and the Mann-Whitney U-test for 2 independent sets. All of those tests were used for the individual hypotheses based on the nature of the particular data included in the given hypothesis. Statistical analysis was carried out in the SPSS 22 environment.

Results

The data set included 68 cases of histologically confirmed basal cell carcinoma of the eyelid. Out of those, in 25 cases it was diagnosed in the UVEA Eye Clinic in Martin in the period from 2018 to 2023, while in 43 cases the BCC was diagnosed in the Department of Ophthalmology of the Central Military Hospital in Ružomberok in the period from 2008 to 2021.

The patients who underwent surgical excision were 14–95 years, with a median of 68 years. There was no statistically significant difference in the number of patients with BCC regarding the age categories of below 65 years and 65+ years of age ($p = 0.140$). A 14-year-old patient had a BCC in the region of the medial canthus on the right side, sized 3x3x2 mm. It was the nodular type (trichoepithelioma-like) of BCC. The resection was carried out with thin margins of less than 2 mm. The histological sample exhibited the Bcl-2 protein expression and positivity, Ber-EP4 positivity and CD10 positivity. No correlation with genetically determined syndromes has been confirmed in this patient.

The BCC incidence slightly prevailed in the female population. The cohort consisted of 29 men (43.9%) and 37 women (56.1%). There was no statistically significant difference between the number of men and the number of women enrolled as patients with BCC ($p = 0.325$).

The largest subgroup of the cohort consisted of the patients with the nodular tumour type - 42.6% of the cohort. By contrast, the smallest subgroup consisted of the patients with the nodular-infiltrative (Table 1).

Table 1 Histological type of BCC in our cohort study.

HISTOLOGICAL TYPE		
	n	**%**
Nodular type BCC†	29	42.6
Cystic type BCC	4	5.9
Infiltrative BCC	14	20.6
Indundibulocystic - trichoepithelioma type BCC	3	4.4
Nodular-cystic type BCC	16	23.5
Nodular-infiltrative type BCC	2	2.9
Total	**68**	**100.0**

† BCC = basal cell carcinoma

The tumour was located on the right side in 54.5%, while in 45.5% the location was on the left side. BCC was most frequently located in the lower eyelid region, representing 44.1 % of the cohort. The smallest subgroup included the patients with a tumour located in near the lateral canthus - 1.5% of the cohort (Figure 1).

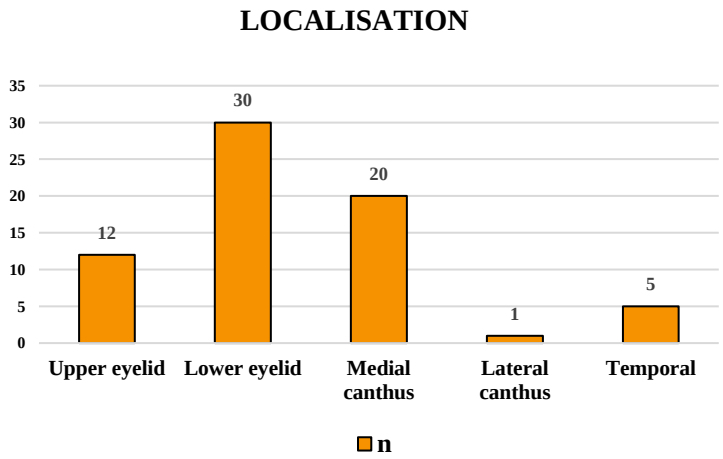

FIGURE 1 Localisation of BCC in our study cohort.

The frequency of a tumour located in the region of the medial canthus is statistically more significant ($p = 0.003$). No statistically significant difference was observed between the frequency of a tumour located in the region of the medial canthus and the frequency of a tumour located in the regions of the upper or the lower eyelids ($p = 0.157$). There was no statistically significant correlation between the presence of recurrence and the tumour location in patients with BCC ($p = 0.165$). The aggressive tumours, as well as the non-aggressive tumours, were most frequently located on the lower eyelid of patients. An interesting fact is that all the

patients with a carcinoma located in the lateral region had the non-aggressive type of tumour.

A relatively extensive surgical excision with the use of skin flaps was carried out in 2 cases. The R1 resection was performed in 8 cases (0.13%). Out of those, in two cases it was sufficient to perform a subsequent re-excision with free margins. In 3 cases, adjuvant HDR brachytherapy was applied at a dose of 12 x 500 cGy; in 3 cases, the re-excision was combined with HDR brachytherapy.

The results showed that the average value of Bcl-2 expression in patients with non-aggressive cancer was 82.65% (Figure 2).

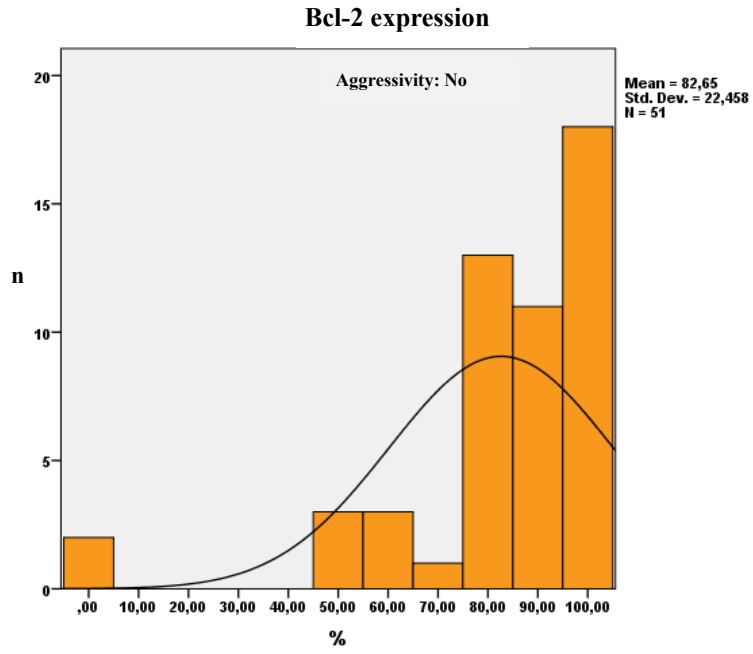

FIGURE 2 The average value of Bcl-2 expression in patients with non-aggressive cancer.

While average value of Bcl-2 expression in patients with aggressive cancer it amounted to 58.42% (Figure 3).

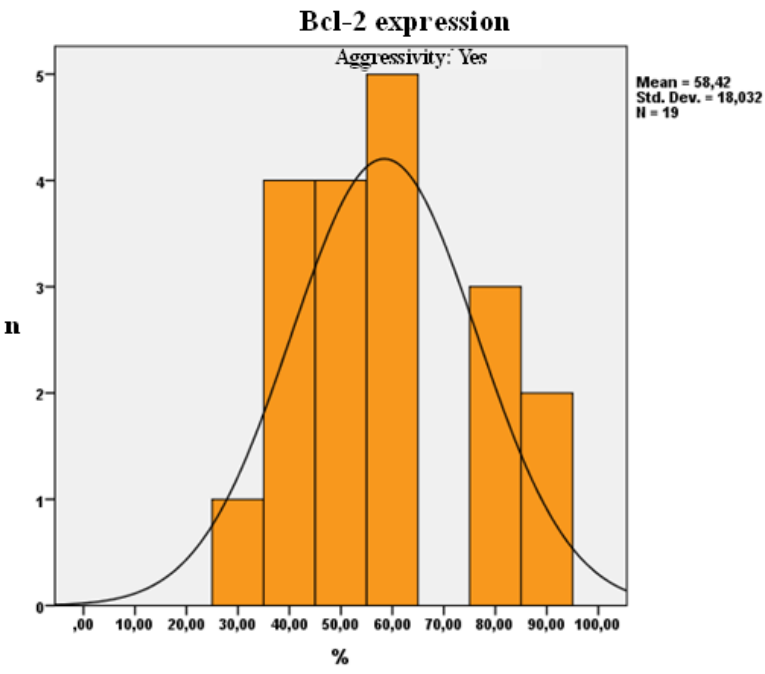

FIGURE 3 The average value of Bcl-2 expression in patients with aggressive cancer.

There was a statistically significant difference in the value of Bcl-2 expression due to the difference in the cancer aggressiveness. The value of Bcl-2 expression in patients with non-aggressive cancer was statistically significantly higher than that observed in patients with aggressive cancer ($p = 0.000$). The Bcl-2 expression was calculated as the total percentage of positive cancerous cells in the carcinoma (Figure 4).

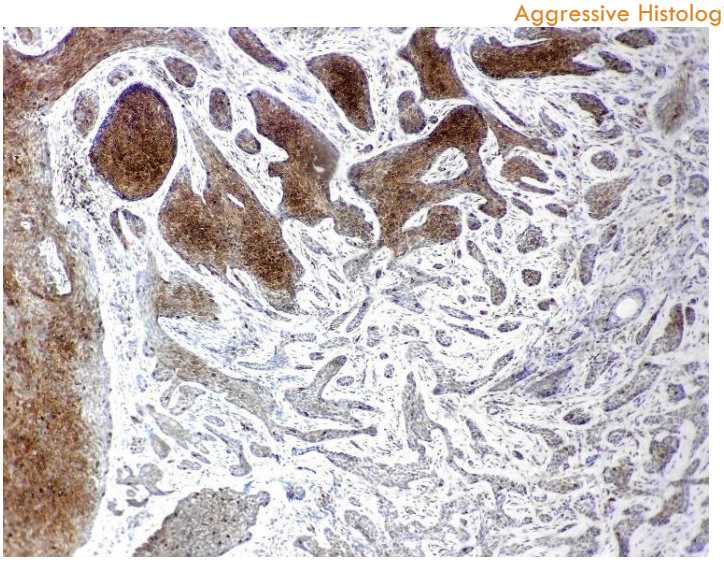

FIGURE 4: Nodular Bcl-2 positivity in the nodular-infiltrative BCC. While the nodular component (the upper left section of the figure) is positive, the infiltrative component (the lower right section of the figure) is almost negative.

The Ki-67 index was calculated as the average percentage of proliferating cancerous cells in the carcinoma (Figure 5).

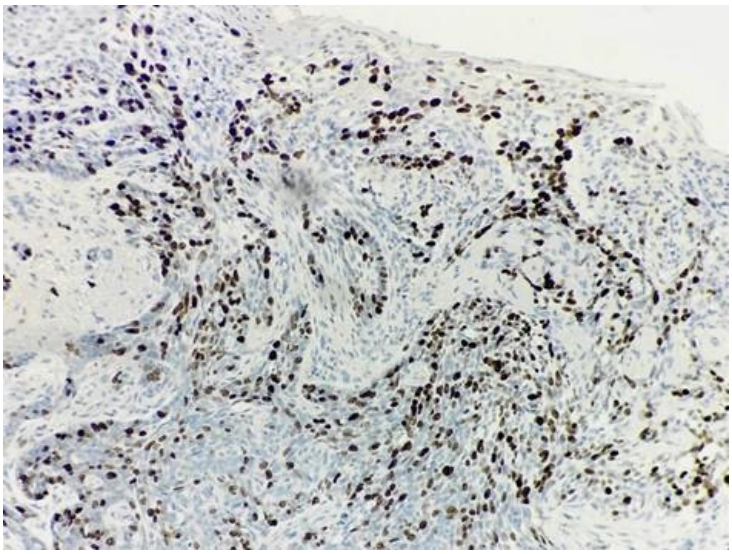

FIGURE 5: Proliferative activity (Ki-67 index) about 40% in nodule-infiltrated BCC.

The results indicated that the average value of Ki-67 expression in patients with non-aggressive cancer amounted to 35.49% (Figure 6).

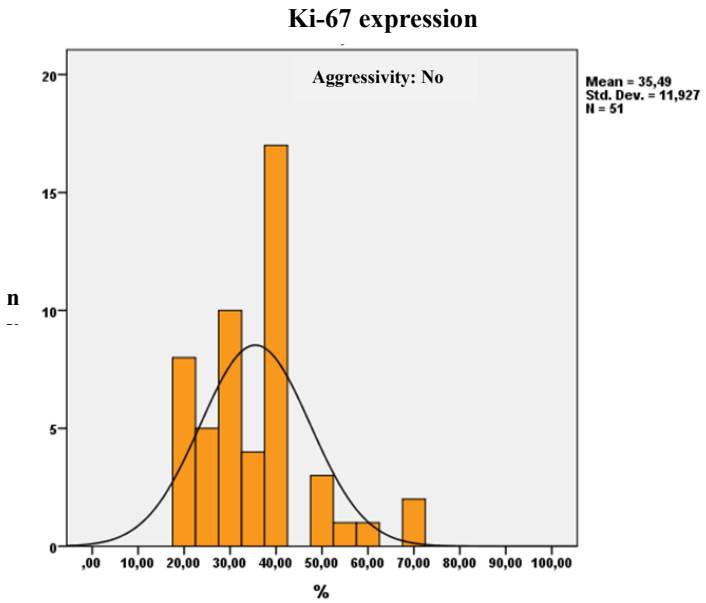

FIGURE 6: The average value of Ki-67 expression in patients with non-aggressive BCC.

While in patients with aggressive cancer the average value of Ki-67 expression was 50% (Figure 7). There was no statistically significant difference in the value of Ki-67 expression about the presence or absence of aggressive cancer ($p = 0.068$).

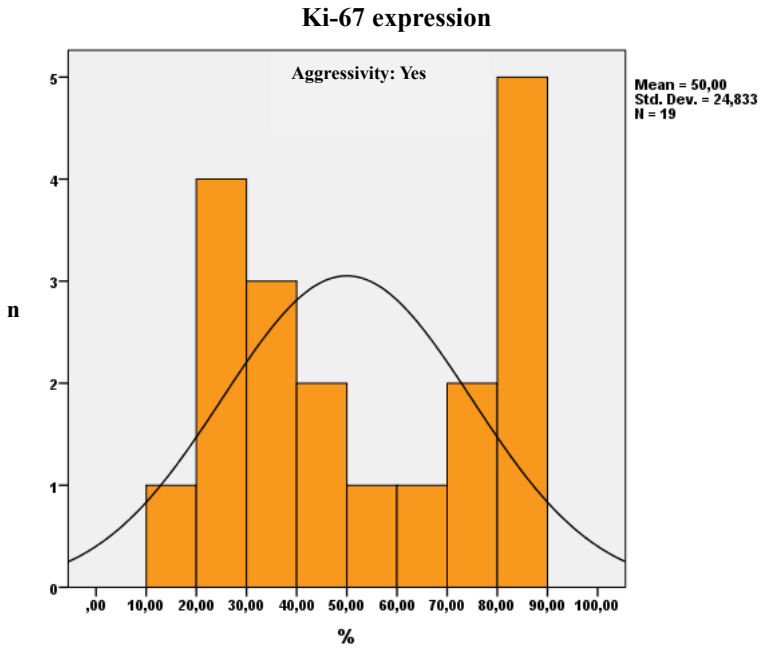

FIGURE 7: The average value of Ki-67 expression in u patients with aggressive BCC.

The apoptotic index was determined based on the semiquantitative evaluation of apoptosis (Figure 8). No statistically significant difference in the apoptotic index was observed with regard to the presence of either aggressive, or non-aggressive cancer ($p = 0.535$). However, the Grade I apoptotic index values were identified in the largest number of samples that were histologically confirmed as non-aggressive cancer. By contrast, in the group of patients with aggressive cancer, the number *Grade I* values and the number of *Grade II* values were identical, while none of the patients with aggressive cancer was confirmed to have *Grade III* AI.

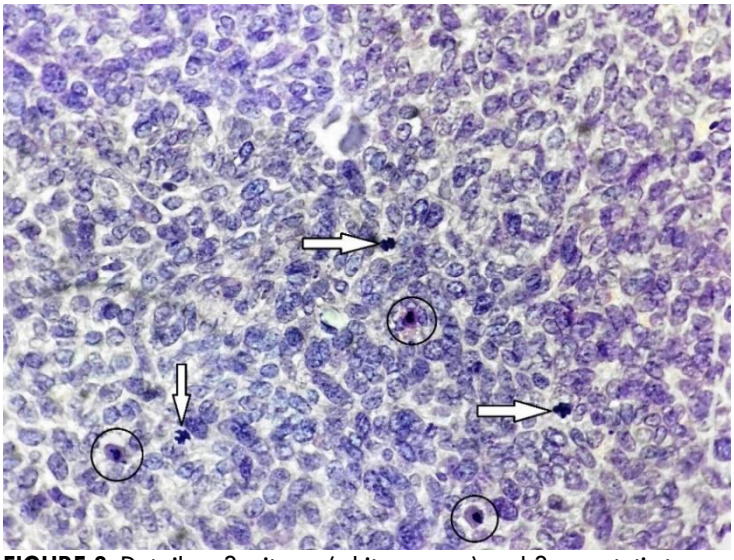

FIGURE 8: Detail on 3 mitoses (white arrows) and 3 apoptotic tumour cells (black rings) in nodular BCC.
Grade I (Gr. I) – the average number of apoptotic tumour cells ranging from 1 to 10 per 10 HPFs (high power fields); i.e. 10 times the microscope objective with a 40x magnification.
Grade II (Gr. II) – the average number of apoptotic tumour cells ranging from 11 to 20 per 10 HPFs (high power fields); i.e. 10 times the microscope objective with a 40x magnification.
Grade III (Gr. III) – the average number of apoptotic tumour cells of ≥ 21 per 10 HPFs (high power fields); i.e. 10 times the microscope objective with a 40x magnification.

In two cases, the orbit was exenterated due to invasive BCC with the infiltration of the surrounding structures (0.03%). In one case, the patient was administered biological therapy. Recurrence was observed during the analysed period in 4 cases – after 2 years on average. As to the expression of prognostic markers, no statistically significant difference was observed.

Discussion

Investigation into a correlation between the growth rate of a malignant tumour of the eyelid and the risk of tumour recurrence after surgical resection might bring some interesting information. Understanding that correlation might bring more light to the dilemma whether the long-growing BCCs acquire a more aggressive phenotype, or

their biological behaviour remains stationary. According to the published studies, it may be assumed that there is a correlation between a higher incidence of BCC and a higher risk of recurrence in individual patients. According to the paper published by Bumpous et al., for patients with multiple BCCs, there was a statistically higher probability of developing BCC and recurrence compared to the patients with a solitary tumour.[10]

Age is a proven risk factor of BCC, with the peak in the 7th decade of life.[11] According to the data in the National Oncology Register of the Slovak Republic, the age structure of Slovak patients indicates a rising trend with ageing. The highest incidence was observed in the age group of 70–74 years.[12] In our study median was 68 years. There was no statistically significant difference regard to the age categories ($p = 0.140$).

Several studies have confirmed a correlation between the gender, the age and the BCC incidence. The incidence is slightly higher in the male population – men represent 61% of cases. The correlation between the patient gender and the risk of BCC recurrence is still controversial.[5,13] Our cohort consisted of 29 men (43.9%).

The tumour location is an important risk factor for the development of BCC. In more than 50% of cases, BCC is located on the lower eyelid; in 30%, it is in the region of the medial canthus; in 15% on the upper eyelid; and in 5% in the region of the lateral canthus. This was the same in our cohort. BCC with the orbital invasion is mainly present in the region of the medial canthus (in 60% of cases, on average) compared to the lower eyelid (30%), the upper eyelid (6%) and the lateral canthus (14%).[14,15] Therefore, there is a significantly higher risk of intraorbital and perineural infiltration in BCC located in the regions of the medial and lateral canthi. Where a lesion afflicts the medial canthus in advanced basal cell carcinoma, there might be a significantly higher need for a mutilating procedure, such as the orbital exenteration.[16] In our study, there was no statistically significant correlation between the presence of recurrence and the tumour location in patients with BCC ($p = 0.165$).

Data on recurrence after a surgical resection of BCC vary, depending on the applied surgical technique. The incidence of recurrence after a surgical resection without the use of the Mohs micrographic surgery or the 'en face' frozen sections ranges from 1.8 to 39%. Recurrence after a BCC excision is facilitated by multiple factors. Its incidence after primary surgery is 1–5% per year. The most frequent cause of recurrence is a thin resection margin, in particular less than 0.3–1 cm from clinically visible margins.[17] The risk of developing non-melanoma eyelid cancer increases if the cancer is also present on/in other body parts. The 5-year cumulative risk of new BCC in patients with at least one previous BCC is 41–45% compared to the 5% risk for the Caucasian population without BCC.[18,19]

The Bcl-2 protein family determines whether a cell dies or survives by controlling mitochondrial apoptosis during the cell division process. Since dysregulation of mitochondrial apoptosis in the cell cycle is an important feature of cancer cells, targeting the protein-protein interactions with the Bcl-2 protein family is a key strategy aimed at seizing control of apoptosis and achieving positive outcomes for the patients with malignancies.[20] Non-Bcl-2 family proteins are emerging as novel apoptosis regulators, and they are being subjected to pharmacological research. The antiapoptotic subfamily includes Bcl-2, Bcl-x, Bcl-w, Mcl-1, and A1. Bcl proteins and carcinogenesis were initially investigated in β-cell lymphoma, while the excessive expression of Bcl-2 was related to neoplastic changes and expansion.[21] Sivrikoz et al. applied the immunohistochemical staining of Bcl-2 in BCC tissues and found out that the reduced levels of Bcl-2 correlated with the aggressive tumour subtypes. Apparently, Bcl-2 protein suppresses cellular death and protects the cells against induced apoptosis.[22] In the study by Puizina-Ivić et al., the authors performed an immunohistochemical analysis of the expression of Bcl-2 protein in histopathological variants of malignant tumours in basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), as well as in a precancerous lesion of actinic keratosis (AK) and in benign seborrheic keratosis (SK). The Bcl-2 expression in BCC forms with a lower risk exhibited the immunoreactivity of the tumour stroma with more intensive staining between the peripheral palisade cells. The morphoeic variant exhibited reduced Bcl-2 expression. None of the SCC tumour samples was Bcl-2 positive. The Bcl-2 protein expression distribution in the low-risk BCC variants showed that the peripherally proliferating cells are protected against apoptosis, which facilitates the tumour growth. In the morpheaform variant, the reduced Bcl-2 expression indicated that in this BCC variant the cell proliferation is higher and in practice exhibits a tendency to induce recurrence and complications related to eradication. The Bcl-2 expression supports the fact that cancerous cells originate in basal keratinocytes.[23] The value of Bcl-2 expression in our study with non-aggressive cancer was statistically significantly higher than that observed in patients with aggressive cancer ($p = 0.000$).

A cell proliferation degree correlates with a rate of tumour growth. A higher degree of proliferation is associated with a lower degree of differentiation, with more aggressive biological behaviour, and a worse prognosis of the malignant disease. Proliferation activity is normally determined immunohistochemically with the use of the antibody against the Ki-67 antigen. Ki-67 antigen is a non-histone protein. It is expressed in the cell nucleus, in particular during the active phase of the cell division cycle, especially in micronuclei. Immunohistochemical examination of the Ki-67 antigen in cancerous cells of a tumour (the Ki-67 index) facilitates the evaluation of their proliferation status. Its value, identified in the histopathological evaluation of a given sample, is one of the basic prognostic markers of biological behaviour of tumours. It has been proved that BCC exhibits a relatively high proliferation activity. The proliferation activity of cancer cells in BCC exhibits an average Ki-67 index of 20–40.6%. Expression of the Ki-67 antigen exhibits significant qualitative and quantitative differences between the individual histological types of BCC.[24]

Multiple studies indicated a correlation between the aggressive behaviour of BCC and a higher degree of cell

proliferation – the Ki-67 proliferation index. However, there have been intensive discussions regarding the prognostic importance of that parameter. Some of the studies provide controversial conclusions. Some authors promote the opinion that the rate of immunohistochemical expression of the Ki-67 antigen is an indicator of BCC aggressiveness and an important prognostic marker, while other authors did not confirm any statistically significant differences in the proliferation activity between the aggressive and non-aggressive BCC types.[25] Authors Healy et al. and Yerebakan et al. observed a higher Ki-67 expression in the primary BCC which eventually recurred. Lower Ki-67 expression was confirmed in BCC without any recurrence.[26] On the other hand, Janisson-Dargaud et al. did not confirm any significant differences in the Ki-67 antigen expression in the primary BCC, without any subsequent recurrence, compared to non-recurrent BCC.[27] In our study, there was no statistically significant difference in the value of Ki-67 in aggressive and non-aggressive tumours ($p = 0.068$).

The apoptotic index (AI) is calculated as the number of apoptotic cells and apoptotic bodies, expressed as the percentage of the total number of tumour cells counted in each case in a histology sample. AI may be regarded as an important prognostic factor of biological behaviour of cancer. However, it is still an unexplained prognostic factor of aggressive histological types of BCC and its recurrence. In their study, Staibano et al. evaluated 60 cases of basal cell skin cancer. In 30 cases, it was the non-aggressive type of basal cell carcinoma (BCC1), while in 30 cases, it was the aggressive type (BCC2). In the tested preparations, BCC1 exhibited a lower apoptotic index (AI) than that observed in BCC2. BCC1: AI: 2.03–10.45% (median: 5.98%); BCC2: AI: 21.91–43.82% (median: 39.82%). It may be assumed that a low apoptotic index in basal cell carcinoma might indicate a good prognosis of a malignant tumour. We noticed no statistically significant difference in the apoptotic index about the presence of aggressive, or non-aggressive cancer ($p = 0.535$). But none of the patients with aggressive cancer was confirmed to have AI=Grade III.

The primary therapeutic modality for BCC is surgical resection. As the tumours are located in the eyelid region, the techniques applied mainly include those that save tissues but increase the risk of recurrence.[4] The eyelid integrity is important for preserving its function, and hence for protecting and maintaining the function of the eyeball. The tumour shape, the distance from the margin, and the tumour diameter are the decisive factors when deciding on the best surgical technique. If the skin lesion covers up to 1/3 of the width of the palpebral fissure, the most appropriate technique is the excision and primary closure. If the skin lesion covers more than 1/3, a different surgical technique should be applied.[27] The simplest and the most common method for tumour excision is the elliptical excision. Recommended procedures exist for the excision of tumorous tissues. The incidence of BCC recurrence after the Mohs micrographic surgery (MMS) is lower than that after the surgical excision (SE). However, MMS is longer and therefore more expensive than SE.[28]

Radiotherapy offers two options of administration – the application of external radiotherapy and brachytherapy in BCC. Radiotherapy plays an important role in the therapy of BCC, SCC, and SGC. Brachytherapy, when compared to surgical resection, has a theoretical advantage as it facilitates the use of a high dose to cover larger skin areas (macroscopic disease, microscopic disease, safety edge) without the necessity of irreversible damage to the surrounding tissues.[29] HDR brachytherapy was applied in 6 cases in our cohort with good effect.

Data on recurrence after the surgical resection of BCC vary, depending on the applied surgical technique. In the available literature published over the last 10 years, the incidence of recurrence without the use of the Mohs micrographic surgery or the perioperative histopathological examination of frozen sections ranged from 1.8% to 39%.[16] The incidence of recurrence after the primary surgery was 1–5% per year. Thinner resection margins – less than 0.3–1 cm from the clinically visible margins, represent a risk factor of BCC recurrence.[17] In our cohort, margins < 2 mm were sufficient, without any connection to recurrence rate ($p = 0.076$). The risk of the development of BCC increases by 41–45% with BCC in the patient's medical history. The histological classification and the subtype of cancer have been clearly established as prognostic factors for invasive and malignant behaviour.[30]

The development of BCC is associated with mutations that lead to changes in the regulation of the Hedgehog (Hh) signalling pathway. Vismodegib is a specific small-molecule inhibitor of the Hedgehog signalling pathway. The signalling of the Hedgehog pathway runs through the Smoothened (Smo) transmembrane protein, leading to the activation and nuclear localisation of transcription factors of the GLI - Glioma-Associated Oncogene and to the induction of the Hedgehog target genes.[31] The approved peroral dose of Vismodegib is 150 mg/day. It is currently approved for the treatment of advanced lesions, as well as metastatic or recurrent BCC. [32,33] A phase 2 pivotal trial (ERIVANCE), conducted with 104 patients, investigated the rate of the BCC response to biological therapy. Local advanced BCC (laBCC) and metastatic BCC (mBCC) exhibited a response in 48% (laBCC) and in 33% (mBCC), while the median duration of response was 7.6–9.5 months. The median survival of patients with mBCC was 33.4 months. Based on the results, it is possible to discuss a neoadjuvant therapy with the Hedgehog inhibitor in locally advanced lesions. However, there are no randomised data proving that the application of a neoadjuvant therapy brings a positive outcome. Out of 15 patients treated with Vismodegib for 3–6 months prior to surgery, recurrence was observed in 1 patient only – after 22 months.[34] We use biological therapy once. Other therapeutic procedures in the treatment are marginal.

Conclusion

Accumulating evidence suggests that members of the Bcl-2 family, apoptotic index, and protein Ki-67 as important regulators of apoptosis, play crucial roles in tumorigenesis, development, and treatment. As demonstrated in this study these biomarkers play a significant role in carcinogenesis of eyelid BCC. Continued research in this area should seek to define the

cellular and molecular targets that control apoptosis and explore its potential for clinical translation.

Conflict of interest statements: The authors declare no conflicts of interest.

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Ethics: Ethics approval for this study was obtained from the Ethics Committee of Central Military Hospital–Teaching Hospital, Ružomberok.

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