ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

# Periocular basal cell carcinoma: demographic, clinical, histological and immunohistochemical evaluation of a series of 39 cases

CLAUDIA FLORIDA COSTEA[1,2)], MIHAELA DANA TURLIUC[3,4)], ANCA SAVA[5,6)], GABRIELA DIMITRIU[2)], GABRIELA FLORENŢA DUMITRESCU[6)], CRISTINA DANCĂ[2)], ANDREI IONUŢ CUCU[4)], CAMELIA MARGARETA BOGDĂNICI[1)], IRINA IULIANA COSTACHE[7)], CĂTĂLIN MIHAI BUZDUGĂ[8)], MANUELA CIOCOIU[9)], DANIELA MARIA TĂNASE[10)], RALUCA ALINA DRAGOMIR[11)], ALEXANDRU CĂRĂULEANU[12)]

[1)] *Department of Ophthalmology, Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*
[2)] *2nd Ophthalmology Clinic, "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital, Iaşi, Romania*
[3)] *Department of Neurosurgery, Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*
[4)] *2nd Neurosurgery Clinic, "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital, Iaşi, Romania*
[5)] *Department of Anatomy, Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*
[6)] *Laboratory of Pathology, "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital, Iaşi, Romania*
[7)] *1st Medical Department, Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*
[8)] *Department of Endocrinology, Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*
[9)] *Department of Pathophysiology, Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*
[10)] *Department of Internal Medicine, Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*
[11)] *Department of Anesthesiology and Oral Surgery, Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*
[12)] *Department of Obstetrics and Gynecology, Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*

## Abstract

*Background*: Basal cell carcinoma (BCC) is the most frequent malignant epithelial tumor of the periocular area. The clinical and histological classification of periocular basal cell carcinoma (pBCC) is essential in order to establish the risk of recurrence and to compare the results of the treatment. Until now, there is no unitary histological classification of pBCC. *Aim*: The aim of this study is to identify the demographic, clinical and histopathological characteristics of adult patients with pBCC, in order to obtain useful data for comparison in other investigations and to identify the histological origin of this eyelid tumor, as there are only hypothesis on this issue. *Materials and Methods*: A descriptive retrospective study was conducted on a series of 39 consecutive patients over the age of 20, who were surgically treated for pBCC in the 2nd Ophthalmology Clinic of the "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital from Iaşi, Romania, over an 11-year period (July 2007–February 2018). The analyzed data were the following: date of resection, patient age at resection, gender, tumor location, histological subtype, and immunohistochemical (IHC) expression patterns of anti-cytokeratin (CK) antibodies (anti-CK AE1/AE3, anti-CK5/6, anti-CK7, anti-CK17, anti-CK19, anti-CK20) and anti-epithelial membrane antigen (EMA) antibody. *Results*: A total of 39 histopathologically diagnosed cases of pBCC were evaluated. The mean age at resection was of 66 years (range: 26–87 years). As for gender, 17 (43.6%) were male patients, with a mean age of 64.84 years (range: 26–78 years) and 22 (56.4%) were female patients, with a mean age of 66.68 years (range: 46–87 years). The pBCC occurred on the right side in 18 (46.2%) cases and on the left in 21 (53.8%) cases. Of all removed specimens, 24 (61.53%) involved the lower eyelid, seven (17.94%) the medial canthus, five (12.82%) the upper eyelid, and three (7.62%) lacked the specification of the site. The most common histological subtype was nodular, accounting for 26 (66.7%) cases. Adenoid BCC was identified in four (10.3%) cases, and morpheaform in one (2.6%) case. Squamous differentiation (basosquamous subtype) was identified in eight (20.5%) cases. The IHC profile of pBCC included: strong immunopositivity for CK AE1/AE3, and CK17 all histological subtypes, and CK5/6 positivity only in squamous differentiation areas. No immunopositivity was identified for CK19, CK20, and EMA IHC staining. *Conclusions*: Our retrospective study indicates that most of pBCCs developed particularly in elderly female patients and tumors were located in the lower eyelid with a left side and lower eyelid preferences. Most of our pBCC cases were histologically classified as nodular subtype, and morpheaform and basosquamous form affected mostly the lower eyelid. pBCC IHC profile showed a strong immunopositivity for CK17, thus suggesting that the origin of this cancer is in the follicular germinative cells. We can conclude that our results showed a demographic, clinical, histological, and IHC profile which seems to be representative for Central and Eastern European countries, maybe due to the same genetic predisposition and environmental factors.

***Keywords*:** periocular basal cell carcinoma, immunohistochemical profile, cytokeratins, eyelid.

## Introduction

Eyelid tumors are by far the most common neoplasms encountered in clinical ophthalmological practice. Basal cell carcinoma (BCC) is still the most common cancer of the eyelid, accounting for 80–90% of all periocular malignancies [1, 2]. Though malignant, BCC comes along with a good prognosis if total removal can be carried out.

There are major differences in the localization and histological subtype of periocular basal cell carcinoma (pBCC) between different geographical areas. In Canada, the most common ocular site of pBCC is the lower eyelid, while in Australia it is the medial cantus. The morpheaform type seems to be significantly higher in Australia compared to Canada. The exposure to ultraviolet (UV) light is greater in Australia, which explains the higher frequency of this subtype of pBCC in the population from this region [3].

BCC or "basalioma" was referred for the very first time from a clinical point of view in 1827 by A. Jacob of Edinburgh [4] and from a histopathological point of view in 1900 by the pathologist E. Krompecher, who reported a malignant epithelioma that had a superficial resemblance to rodent ulcer and took a position in what concerns the histological differences between cornified superficial carcinoma and adenocarcinoma. He named it "carcinoma epitheliale adenoidea" (adenoid epithelial carcinoma) [5].

In 1901, the Hungarian dermatologist Ludwig Nékám used the term "basalioma adenoids cysticum" [6]. Later on, in 1903, Krompecher stated that this tumor derived from the basal cell layer of the epidermis and named it "der Basalzellenkrebs" (BCC) [7].

Prolonged exposure to UV light is the most important risk factor. Typically, BCC emerges on sun-exposed parts of the body, such as the face, neck, and head [8]. Patients with BCC are usually over 50 years, but in recent years, there has been an increase in the number of younger patients suffering from this type of cancer, due to prolonged exposure to sunrays or sunbeds. In terms of etiopathology, certain authors believe that BCC develops from the progenitor cells residing in the intra-follicular epidermis and upper infundibulum [9], but the precise origin of this type of cancer has yet to be fully discovered [10]. The clinical assessment and histological classification of pBCC is important in establishing the risk of recurrence and in comparing the results of the treatment. The histopathological classification of BCC must be based on the tumor growth pattern, histological differentiation, but it must also be correlated with the clinical behavior [11].

Up to the present days, there is no homogeneous histological classification of pBCC. The most complete classification should take into account both the growth pattern and the differentiation rate of tumor cells. Until now, 26 histopathological subtypes have been described [12]. According to the risk of recurrence, the pBCC are classified into low-risk tumors, which include the nodular, adenoid and basosquamous subtypes, and high-risk tumors, which include the morpheaform subtype [13].

The aim of our study was to identify the demographic, clinical, histopathological, and immunohistochemical (IHC) characteristics of pBCC in order to obtain some useful data for comparison in other investigations, a possible profile of this tumor in our region, and also to identify the histological origin of this eyelid tumor as there are only hypothesis on this issue.

## Materials and Methods

A retrospective demographic, clinical, histological, and IHC review was conducted for all periocular (upper and lower eyelid, medial and lateral canthus) excision specimens diagnosed as pBCC in the Department of Pathology of the "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital, Iaşi, Romania, over an 11-year period (from July 2007 to February 2018). All the cases were selected from medical records and Pathology database. The analyzed data were the following: date of tumor resection, patients' gender and age at tumor resection, tumor location, and histological tumor subtype. The management of the study was conducted in full compliance with the ethical principles and informed consent has been obtained from all the patients.

Tumor resection was performed in the 2nd Ophthalmology Clinic of the same Hospital, and the surgical specimens were fixed in 4% neutral-buffered formalin and embedded in paraffin, according to routine procedures. Paraffin tissue blocks were cut into 3 μm thick sections and then stained with Hematoxylin–Eosin (HE).

After all this data were collected, representative tissue blocks of all patients were then sectioned at a thickness of 3 μm, dried for one hour at 65°C before pretreatment procedure of deparaffinization and rehydration. Epitope retrieval was realized in citrate buffer, pH 6.5, in water bath at 95°C for 30 minutes. Before staining the sections, endogenous peroxidase activity was blocked. We used the following antibodies: anti-cytokeratin (CK) AE1/AE3 (Thermo Fisher Scientific, USA), CK5/6 (DAKO, Denmark), CK7 (DAKO, Denmark), CK17 (DAKO, Denmark), CK19 (DAKO, Denmark), anti-CK20 (Thermo Fisher Scientific, USA), and anti-epithelial membrane antigen (EMA) (DAKO, Denmark) (Table 1).

**Table 1 –** ***The antibodies used for IHC staining of pBCC and the expression pattern for the CKs and EMA in normal skin***

| Antibody | Manufacturer | Clone | Antigen retrieval | Class | Dilution | Labeling | Cellular localization |
|---|---|---|---|---|---|---|---|
| Anti-CK AE1/AE3 | Thermo Fisher Scientific | AE1/AE3 | Citrate, pH 6 | Monoclonal mouse anti-human CK AE1/AE3 | RTU | Epithelial cells | Cytoplasmic |
| Anti-CK5/6 | DAKO | D5/16 B4 | Citrate, pH 6 | Monoclonal mouse anti-human CK5/6 | 1:100 | Epidermis (basal and parabasal cells), outer root sheath of hair follicle, glandular epithelia (sebaceous gland, sweat gland) | Cytoplasmic |
| Anti-CK7 | DAKO | OV-TL 12/30 | Citrate, pH 6 | Monoclonal mouse anti-human CK7 | 1:100 | Sebaceous gland (sebocytes and undifferentiated cells) and sweat glands (all parts except secretory cells) | Cytoplasmic |
| Anti-CK17 | DAKO | E3 | Citrate, pH 6 | Monoclonal mouse anti-human CK17 | 1:40 | Hair follicle (only in the bulge and follicular germinative cells), sebaceous gland (undifferentiated cells), and sweat gland (only in secretory cells) | Cytoplasmic |

| Antibody | Manufacturer | Clone | Antigen retrieval | Class | Dilution | Labeling | Cellular localization |
|---|---|---|---|---|---|---|---|
| Anti-CK19 | DAKO | RCK108 | Citrate, pH 6 | Monoclonal mouse anti-human CK19 | 1:100 | Basal cells on the external root sheath of hair follicles) and sweat gland (only secretory cells) | Cytoplasmic |
| Anti-CK20 | Thermo Fisher Scientific | EP23 | Citrate, pH 6 | Polyclonal rabbit anti-human CK20 | RTU | Merkel cells from basal epidermal layer of the skin | Cytoplasmic |
| EMA | DAKO | E29 | Citrate, pH 6 | Monoclonal mouse anti-human EMA | 1:50 | Glandular and ductal epithelial cells of eccrine and apocrine sweat glands | Cell membrane and cytoplasmic |

IHC: Immunohistochemical; pBCC: Periocular basal cell carcinoma; CK: Cytokeratin; EMA: Epithelial membrane antigen; RTU: Ready-to-use.

After incubation, the reaction was visualized with the EnVision FLEX Detection Kit (DAKO, Denmark), for CK5/6, CK7, CK17, CK19, and EMA, or with UltraVision™ Quanto Detection System Horseradish Peroxidase (HRP) 3,3'-Diaminobenzidine (DAB) (USA), for anti-CK AE1/AE3, and anti-CK20, using DAB chromogen as a substrate. Sections were counterstained with Mayer's Hematoxylin for nuclear counterstaining. The reaction was considered positive only when a brown cytoplasmic staining was detected.

## Results

From 2007 to 2018, a total of 39 adult patients with histopathologically proven pBCC were identified. The characteristic features of patients and of their tumors are presented in Table 2.

**Table 2 –** ***The characteristic features of the pBCCs from our series***

| Characteristics of the tumor | Frequency No. (%) |
|---|---|
| *Localization* | |
| ▪ Lower lid | 24 (61.5%) |
| ▪ Medial canthus | 7 (17.94%) |
| ▪ Upper lid | 5 (12.82%) |
| ▪ Lateral canthus | – |
| ▪ Unspecified | 3 (7.62%) |
| *Side* | |
| ▪ Right | 18 (46.2%) |
| ▪ Left | 21 (53.8%) |
| *Gross pathology* | |
| ▪ Vegetating lesion | 10 (25.64%) |
| ▪ Nodulo-ulcerative (rodent ulcer) | 29 (74.35%) |
| *Histological subtype* | |
| ▪ Nodular | 26 (66.7%) |
| ▪ With squamous differentiation | 8 (20.5%) |
| ▪ Adenoid | 4 (10.3%) |
| ▪ Morpheaform | 1 (2.6%) |

pBCC: Periocular basal cell carcinoma.

The patients' mean age at resection was of 66 years (range: 26–87 years). Related to gender, 17 (43.6%) were male patients, with a mean age of 64.84 years (range: 26–78 years), and 22 (56.4%) were female patients, with a mean age of 66.68 years (range: 46–87 years). The female:male (F:M) ratio was 1.78. Most patients were included in the 70–79 years age group (13 cases, 33.33%). Twenty-four (61.53%) pBCCs involved the lower eyelid, seven (17.94%) the medial canthus, five (12.82%) the upper eyelid, while three (7.62%) did not have a specified location. The pBCC occurred on the right side in 18 (46.2%) cases and on the left in 21 (53.8%) cases (Table 2).

A vegetating lesion was encountered in 10 (25.64%) cases and a nodulo-ulcerative (rodent ulcer) lesion in 29 (74.35%) cases (Table 2; Figure 1, a and b).

The most common histological subtype was the nodular one, accounting for 26 (66.7%) cases (Figure 2, a and b; Figure 3, a and b). Adenoid subtype was identified in four (10.3%) cases (Figure 4, a and b; Figure 5), and the morpheaform subtype in one (2.6%) case (Figure 6). The pBCC with squamous differentiation was identified in eight (20.5%) cases (Table 2; Figure 7, a and b).

Correlating patients' gender with their tumoral histological subtype, we found out that nodular pBCC emerges almost in the same percentages both in male and female patients, even though there is a slight predominance in female patients (14 female cases *vs.* 12 male patients). Instead, morpheaform pBCC developed only in one male patient. pBCC with squamous differentiation affected mostly female patients (five female patients *vs.* three male patients), and adenoid pBCC mostly affected female patients (Figure 8).

We found out a predominance of the nodular pBCC in the left eye (14 cases), followed by pBCC with squamous differentiation (five cases). Nodular pBCC prevailed in the right eye (12 cases), but adenoid pBCC and basosquamous pBCC occurred in equal number (three cases) (Figure 9).

Nodular pBCC came out in 16 cases located in the lower eyelid. The unique case of morpheaform pBCC developed only in the left lower eyelid. The basosquamous pBCC was diagnosed mainly on the lower eyelid (five cases).

In all the cases, the pre-operative diagnostic was an eyelid tumor. In one single case, BCC was associated with a chalazion, both identified on the histopathological examination (Figure 4).

The IHC profile of pBCC included: strong immunopositivity for CK AE1/AE3 (Figures 10 and 11) and CK17 (Figure 12) in all histological subtypes, and CK5/6 positivity (Figure 13) only in squamous differentiation areas of pBCC. No immunopositivity was identified for CK7 (Figure 14), CK19 (Figure 15, a and b), CK20 (Figure 16), and EMA (Figure 17, a and b) in all pBCCs we have studied.

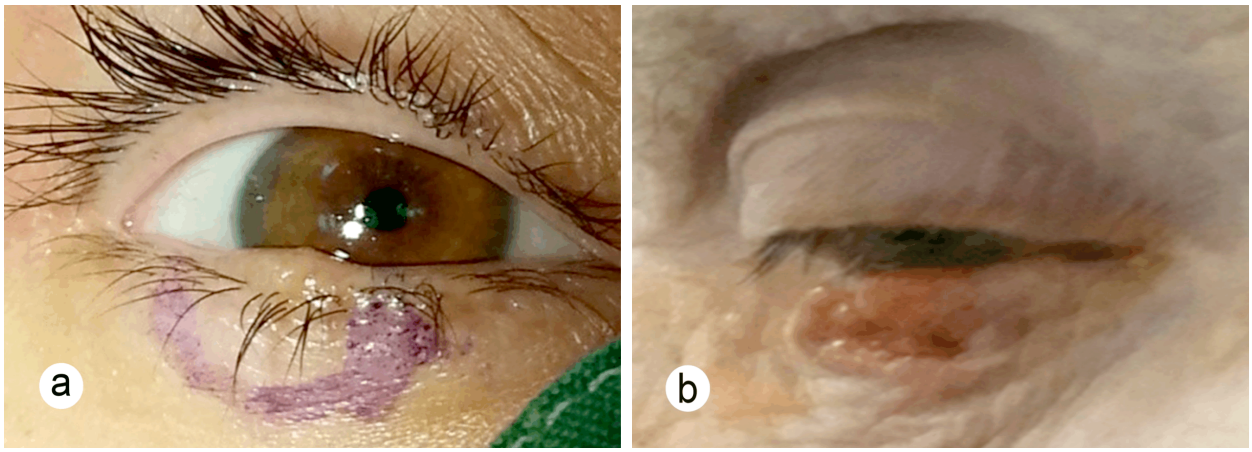


**Figure 1 –** ***Clinical image of pBCC: (a) Vegetating lesion; (b) Nodulo-ulcerative (rodent ulcer) lesion. pBCC: Periocular basal cell carcinoma.***

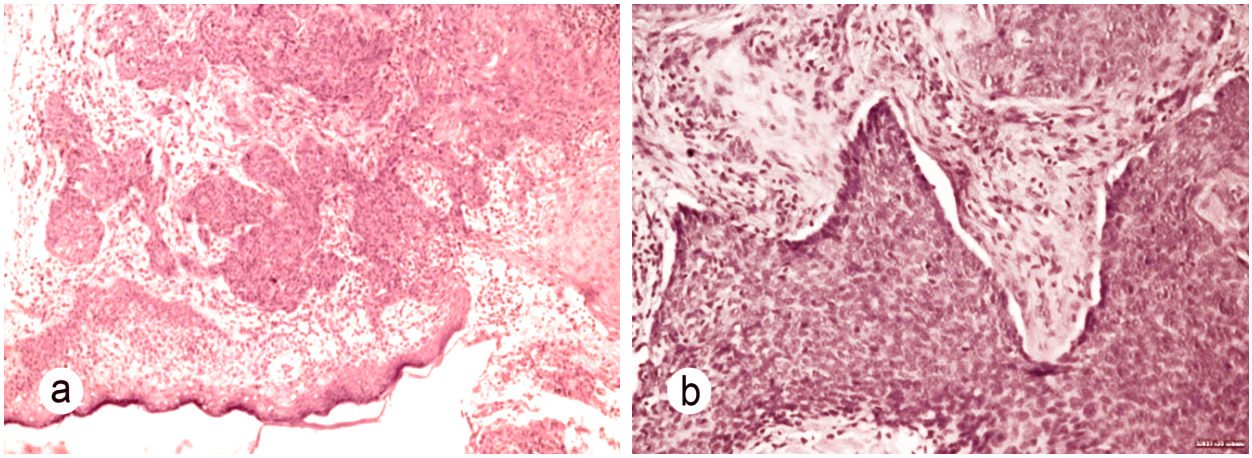


**Figure 2 –** ***Nodular pBCC: (a) The tumor maintained epidermal connection, but invaded the dermis; (b) Higher magnification of the same case revealed tumoral masses consisting of basaloid cells, with peripheral palisading and artefactual clefts. HE staining: (a) ×100; (b) ×200. pBCC: Periocular basal cell carcinoma.***

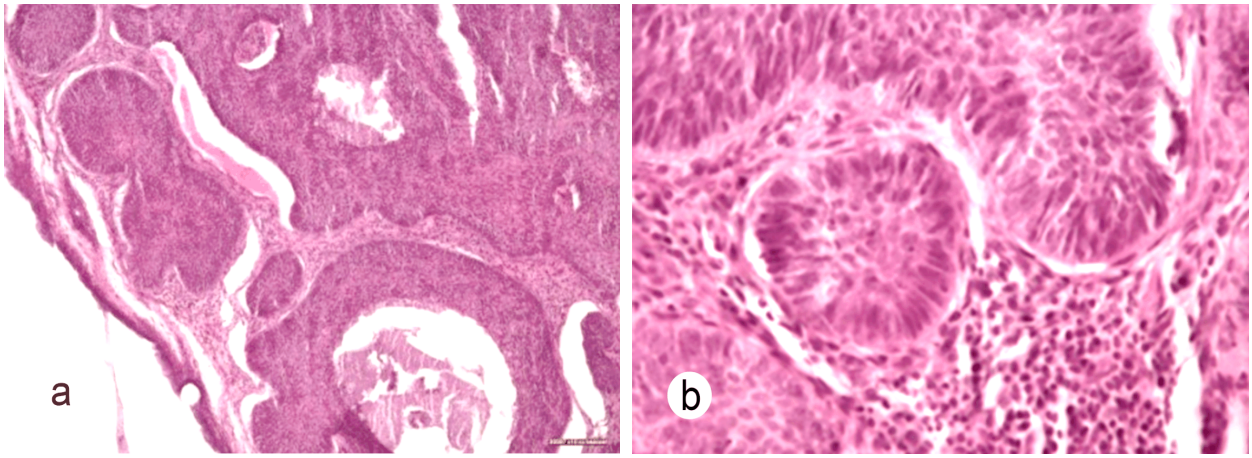


**Figure 3 –** ***Nodular pBCC: (a) Basophilic tumoral nodules infiltrated the dermis; (b) The tumoral island presented a peripheral layer with a palisade arrangement, and was encircled by an artefactual retraction space between the tumor and the stroma – a strong chronic inflammation could be seen in the tumoral stroma. HE staining: (a) ×100; (b) ×400. pBCC: Periocular basal cell carcinoma.***

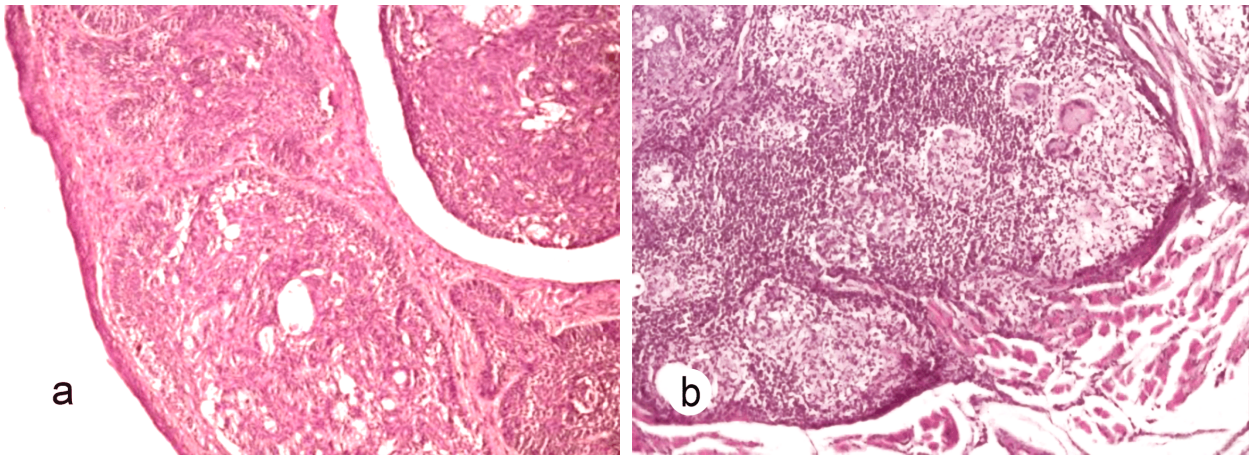


**Figure 4** – ***pBCC with areas of adenoid differentiation and an associated chalazion: (a) Islands of tumoral basal cells showing some adenoid differentiation invaded the dermis; (b) In the nearby dermis, there were multiple foci of granulomatous inflammation with micro-abscesses and multinucleated giant cells. HE staining: (a and b) ×100. pBCC: Periocular basal cell carcinoma.***

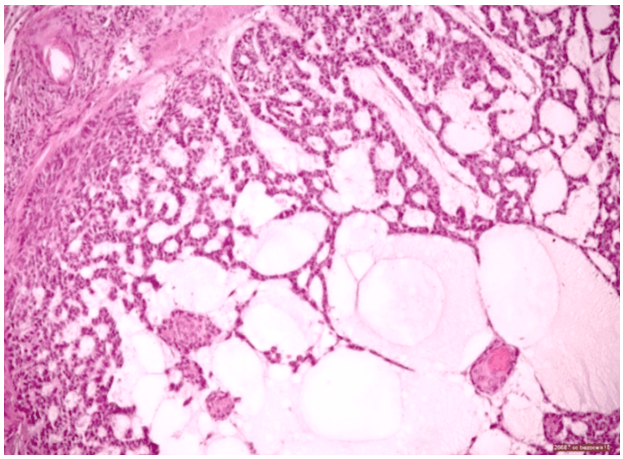

**Figure 5** – ***Adenoid pBCC showing tubular/glandular differentiation inside the tumoral masses. HE staining, ×100. pBCC: Periocular basal cell carcinoma.***

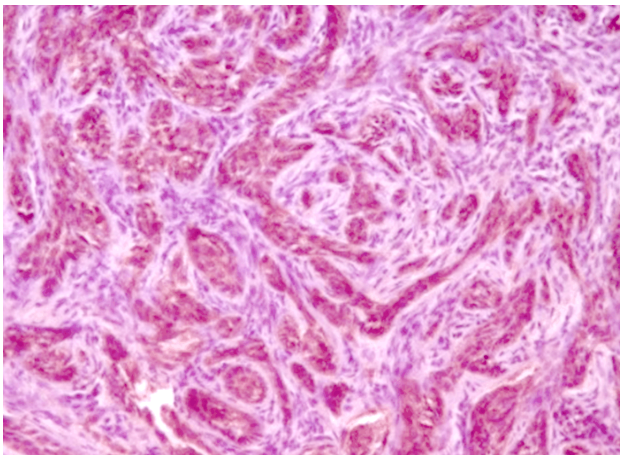

**Figure 6** – ***Morpheaform pBCC: angulated and narrowed tumoral trabeculae growing in an infiltrative pattern were associated with collagenized stroma. Anti-CK AE1/AE3 antibody immunostaining, ×200. pBCC: Periocular basal cell carcinoma; CK: Cytokeratin.***

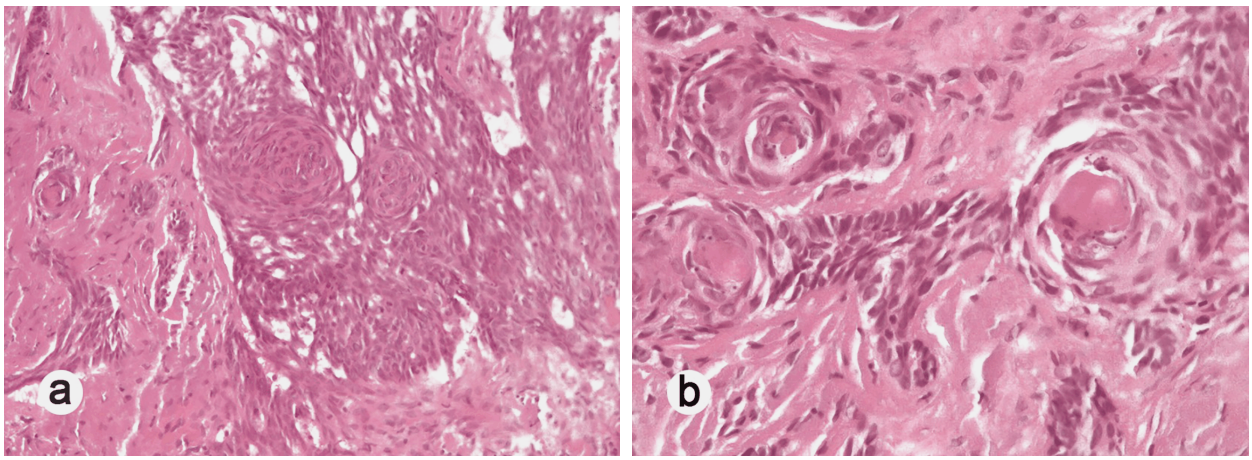


**Figure 7** – ***pBCC with squamous differentiation showed solid or trabecular growth pattern containing central foci with pronounced squamous differentiation (a) and even keratinization (b). HE staining: (a) ×200; (b) ×400. pBCC: Periocular basal cell carcinoma.***

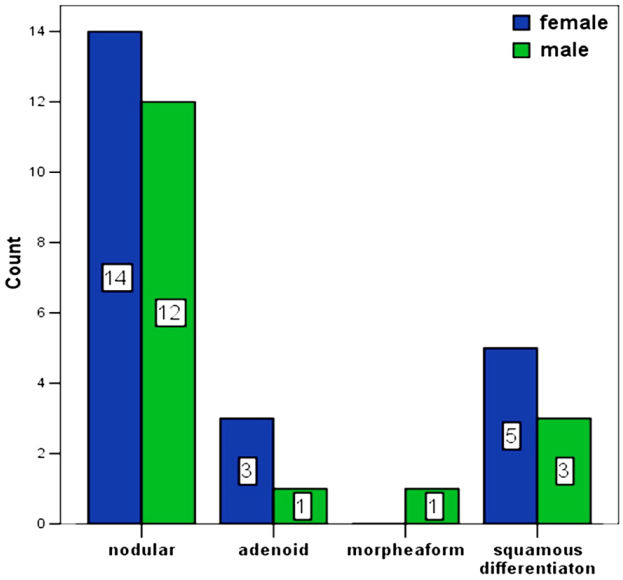


**Figure 8** – ***Distribution of pBCCs according to their histological subtypes. pBCC: Periocular basal cell carcinoma.***

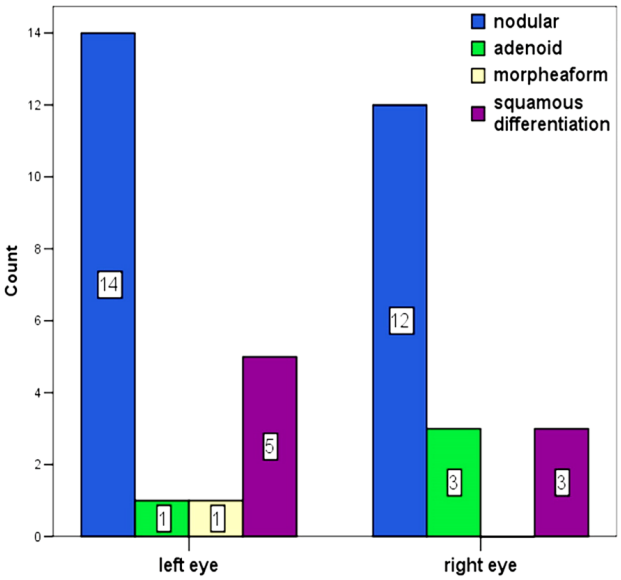


**Figure 9** – ***pBCC side location according to their histological subtypes. pBCC: Periocular basal cell carcinoma.***

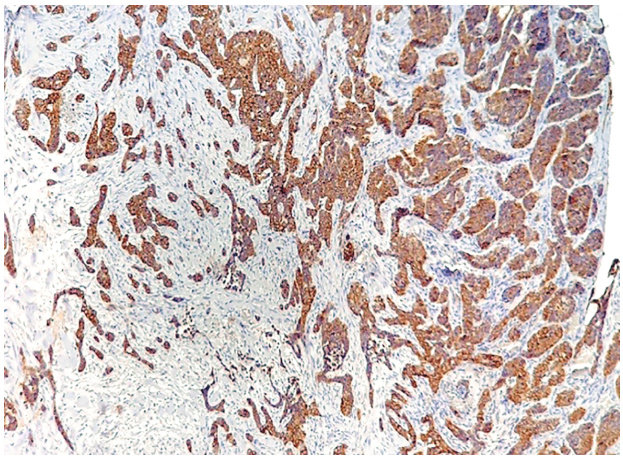

**Figure 10** – ***pBCC with very intense CK AE1/AE3 expression. Anti-CK AE1/AE3 antibody immunostaining, ×100. pBCC: Periocular basal cell carcinoma; CK: Cytokeratin.***

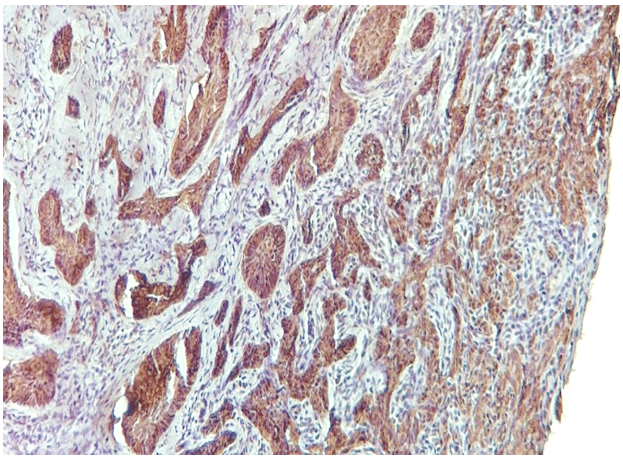

**Figure 11** – ***pBCC showing strong immunopositivity for CK AE1/AE3. Anti-CK AE1/AE3 antibody immunostaining, ×200. pBCC: Periocular basal cell carcinoma; CK: Cytokeratin.***

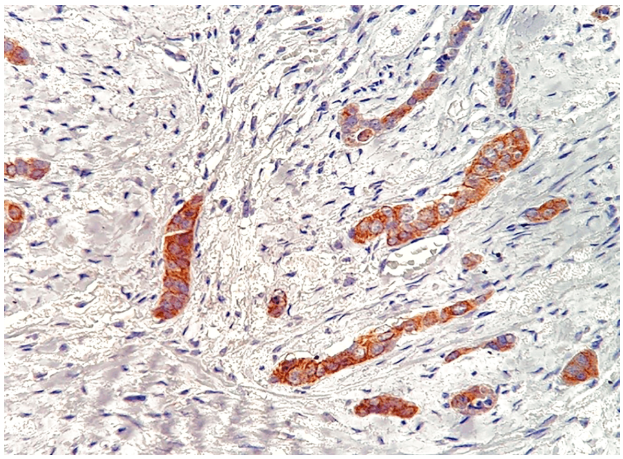

**Figure 12** – ***pBCC with very intense CK17 expression. Anti-CK17 antibody immunostaining, ×400. pBCC: Periocular basal cell carcinoma; CK: Cytokeratin.***

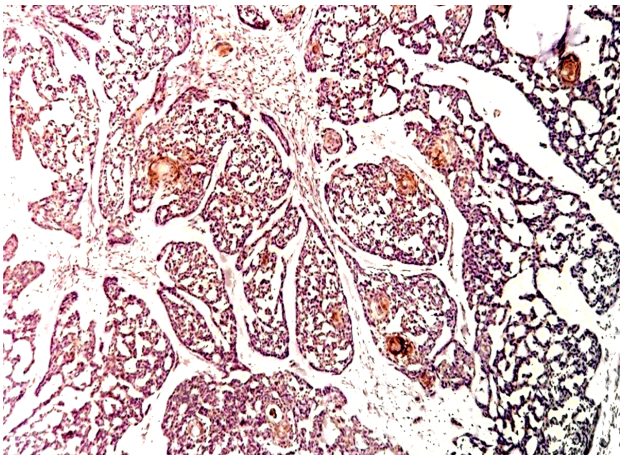

**Figure 13** – ***Adenoid pBCC with strong CK5/6 expression of tumoral squamous differentiation areas. Anti-CK5/6 antibody immunostaining, ×100. pBCC: Periocular basal cell carcinoma; CK: Cytokeratin.***

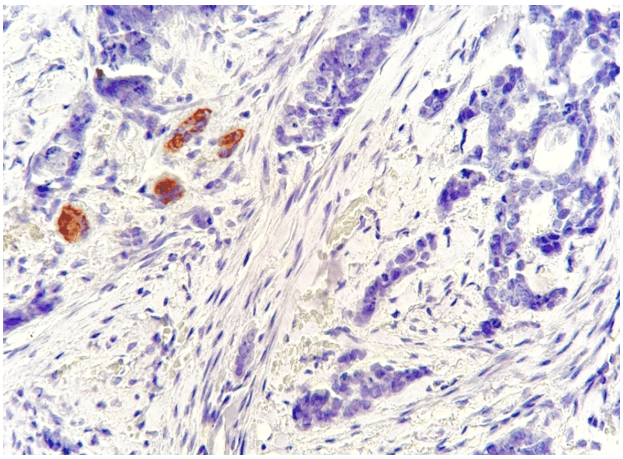

**Figure 14 –** ***Strong CK7 immunopositivity of intratumoral remnants of secretory cells of sweat glands, but no staining of pBCC was obtained with this marker. Anti-CK7 antibody immunostaining, ×400. CK: Cytokeratin; pBCC: Periocular basal cell carcinoma.***

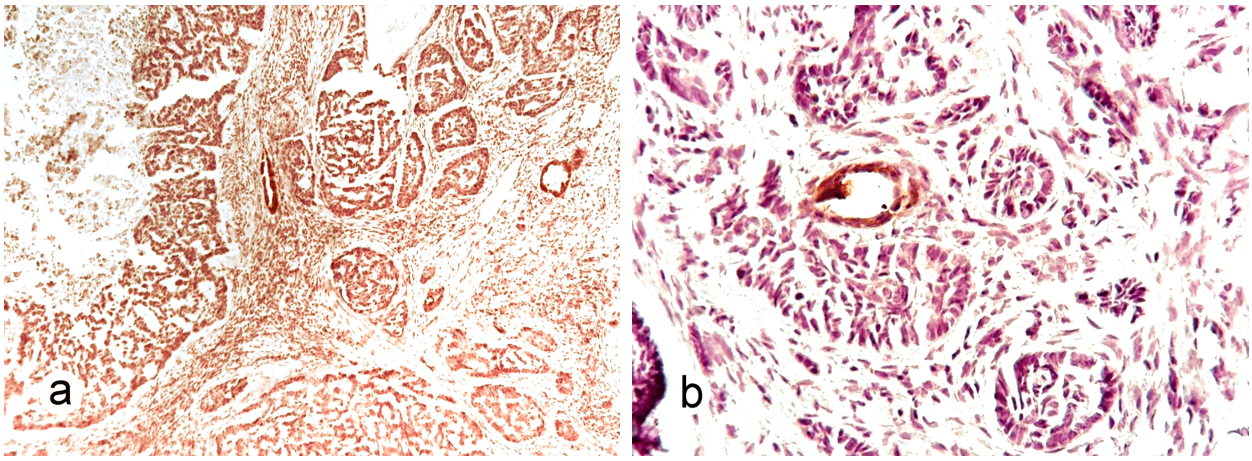


**Figure 15 –** ***Strong CK19 immunopositivity of intratumoral remnants of secretory cells of sweat glands, but there was no staining of pBCC. Anti-CK19 antibody immunostaining: (a) ×100; (b) ×400. CK: Cytokeratin; pBCC: Periocular basal cell carcinoma.***

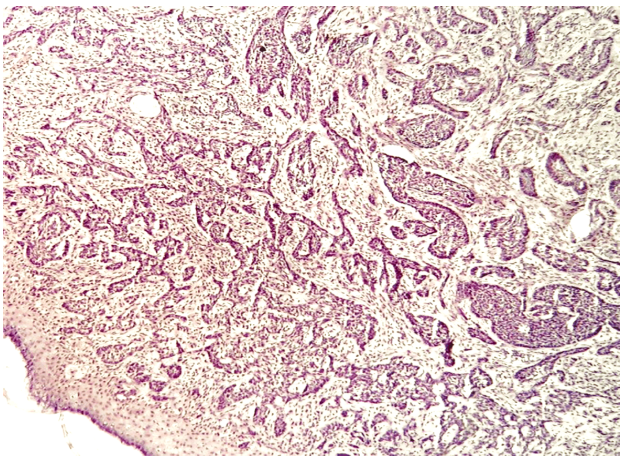

**Figure 16 –** ***pBCC did not expressed immunoreactivity with anti-CK20 antibody immunostaining, ×100. pBCC: Periocular basal cell carcinoma; CK: Cytokeratin.***

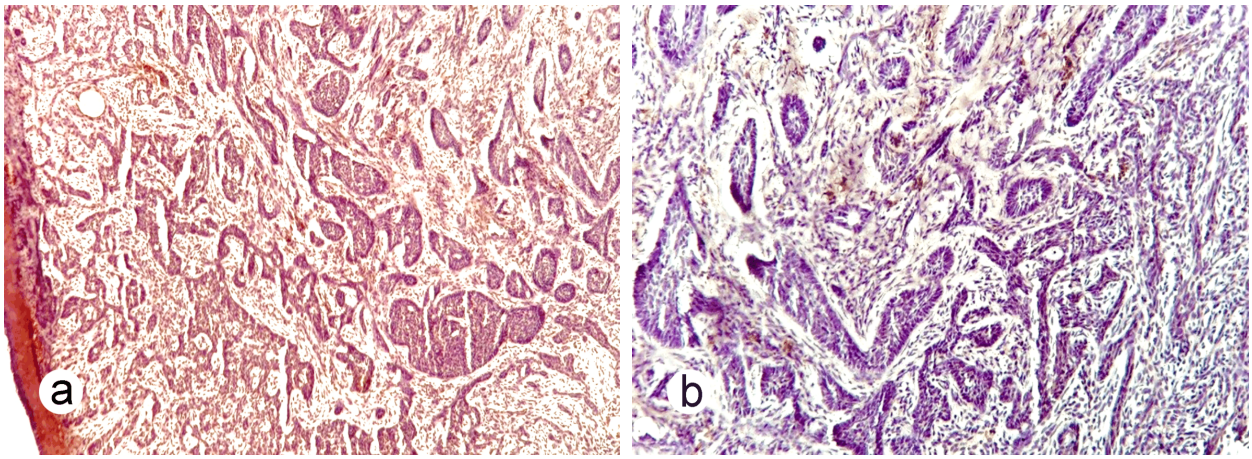


**Figure 17** – ***pBCCs did not express immunoreactivity with anti-EMA antibody immunostaining: (a) ×100; (b) ×200. pBCC: Periocular basal cell carcinoma; EMA: Epithelial membrane antigen.***

## Discussions

Literature reports that BCC affects mostly adults, hence the mean age at resection is around 68 years [14] and the highest incidence is found among patients aged 70–74 [15]. In Romania, BCC predominate in patients over 50 (almost 80% of cases), in males (more than 60%), being localized mostly in the head and neck regions (83%) [16]. Almost 90% of our patients were older than 50, most of them being in their 8$^{th}$ decade of life. In Asian countries, pBCCs develop at a younger age probably due to a longer exposure to UV light per day. Thus, Hui *et al.* (2017) reported the highest incidence in patients aged 51–60 [17]. In our series, we identified only 10% patients with pBCCs that were younger than 50, the youngest being a male aged 26.

Some authors reported a slight predominance of pBCC in male patients [14], while others identified a prevalence of these cancers among the female patients [17], or even found the same gender distribution [15]. We identified a higher prevalence of pBCC in female patients in our series.

Literature highlights the fact that the most common site for pBCC is the lower eyelid (52.7–63.1%), followed by the medial canthus (29.8%), upper eyelid (7.5–5.7%) and lateral canthus (29–1.4%) [2, 14, 18, 19]. Our study revealed almost the same pattern. Interestingly enough, Arlette *et al.* (1998) reported that almost 50% of their pBCCs were located in the medial canthal area, and only one third of cases occurred in the lower eyelid [20].

Some authors found that pBCCs are located mostly on the right side, but others reported a higher incidence on the left side [15]. According to our study, pBCC developed more frequently on the left, yet the difference was not a major one in terms of statistics.

The clinical evolution of eyelid BCC is one of a slow, painless tumor. Patients presented to the ophthalmologist after an average period of three years. This delayed examination results in an orbital invasion [21].

However, the clinical presentation of pBCC can be variable. It may present itself as a papulonodular lesion with a pearly translucent edge, as an ulcerated destructive lesion (rodent ulcer), a plaque with variable amount of induration, an erythematous plaque with visibly dilated vessels, or as a partly cystic nodule [22]. In most of the cases, we found a nodular-ulcerative lesion and this could mean that the patients presented in our Clinic in an advanced stage of their tumor evolution.

From a histopathological point of view, pBCC consists of islands of neoplastic epithelial cells, displaying a prominent palisading in the periphery and a haphazard arrangement in the center. Typically, the tumor retains a certain connection with the surface of the epidermis. Mitoses and single-cell apoptosis are usually present and may be prominent. The tumoral islands are surrounded by a fibrous stroma and there is an artefactual retraction around them, which is characteristic for this type of tumor. Intratumoral mucin may form large pools or cystic spaces. Their stroma also displays variable proportions of lymphocytic inflammation, prominent fibroblasts, and collagen thickening [23].

Literature reveals a considerable variability in the morphological spectrum of the BCCs, and, as a result, a number of histopathological subtypes have been defined, including superficial, nodular (solid), micronodular, infiltrative, fibroepithelioma, basosquamous, and keratotic types. BCCs were also classified as undifferentiated and differentiated, with the latter showing differentiation towards the hair infundibulum, sebaceous glands, or apocrine and eccrine glands (adenoid). There can be mixed patterns as well [22, 24–26].

The morpheaform and basosquamous subtypes of pBCC are rare, but more aggressive, having a higher rate of residual positive margins after excision, as well as a larger risk of recurrence and metastasis. The morpheaform pBCC has a particularly higher recurrence rate and it is more likely to determine perineural invasion. Aggressive-growth BCC variants, including micronodular, infiltrative, morpheaform, and basosquamous share features of increased cell necrosis, mitotic activity, and stromal proliferation, with a decreased demonstration of stromal retraction, deeper growth, and less circumscription [27, 28].

In the present series, irrespective of histological subtype, most of the cases (66.7%) were nodular pBCCs, being consistent with other studies [15].

The second in rank (the fifth part of all cases) was pBCC with squamous differentiation, which has the characteristics of both the BCC and squamous cell carcinoma (SCC), being considered an aggressive type

of cancer. Even though the morpheaform BCC is the most aggressive subtype, it was the rarest among our cases (less than 3%).

We found out some interesting facts that were not reported until now. In our series, the unique case of morpheaform pBCC developed only in the left lower eyelid. Also, the pBCC with squamous differentiation was diagnosed mainly on the lower lid, *i.e.*, the area most affected by eyelid squamous carcinoma. These data suggest that lower eyelid is associated with the most aggressive histological types of skin carcinomas.

We also found out some other data that were not highlighted by other studies, *i.e.*, the morpheaform pBCC developed only in male patients, but pBCC with squamous differentiation affected mostly female patients.

Researchers from Craiova, Romania, realized a study similar to ours and found out almost the same results as ours. They identified nodular pBCCs in 70% of their cases, adenoid BCCs in almost 20% of cases, cystic BCCs in 12.5%, and less than 10% were morpheaform BCCs [29].

Many studies searched for an IHC panel to distinguish BCC from basaloid SCC of the head and neck. In our series, all histopathological subtypes of pBCC expressed strong immunopositivity for anti-CK AE1/AE3 antibody. Other authors obtained the same results [30]. Other studies found out that BCC cases showed no staining for EMA antibody, in contrast to SCCs that expressed in more than 80% of cases a strong positivity for this marker [31]. We also found out that EMA has no expression in pBCC and this fact makes the distinction of basal and SCCs of the eyelid skin to be readily achieved with routine immunohistochemistry using this epithelial antibody [32].

Numerous studies highlighted the fact that CK17 is a useful marker in the identification of BCC, especially the morpheaform subtype that must be distinguished from other adnexal neoplasms, for example desmoplastic trichoepithelioma [33]. Our research proved the fact that anti-CK17 antibody is a reliable marker also for pBCC as we obtained a strong immunopositivity for our cases.

In our study, pBCC did not express CK19 positivity. With this antibody, we identified only remnants of sweat glands entrapped into the tumors. As such, pBCC seems to have its origin in UV light induced mutations on outer sheath of the hair follicle cells as some other authors have also pointed out [34]. Alessi *et al.* (2008) studied the CK profile of BCC using monoclonal antibodies against 12 CKs in order to identify the origin of this tumor as some hypothesis pointed out to follicular matrix cells or from follicular outer root sheath cells [34]. They found out CK5 and CK17 positivity in all the BCCs studied; CK7, CK8, CK18, and CK19 expressed variable positivity (in 30/52, 33/52, 42/52, and 14/52 BCCs, respectively); CK14 showed negativity in almost all the BCCs studied. Their study suggests that BCC expresses a differentiation toward follicular outer root sheath cells and, in most cases, also toward the glandular components of the pilosebaceous-apocrine unit. Our study proved that CK profile of pBCC consisted in CK AE1/AE3 and CK17 intense immunopositivity, and CK5/6 immunopositivity only in squamous differentiation of pBCC. All the other CKs (CK7, CK19, CK20), and also EMA did not express immunopositivity in pBCC.

Romanian researchers from Craiova identified another IHC profile for eyelid BCCs [29]. They found out a moderate expression of B-cell lymphoma-2 (Bcl-2) marker in the nodular type of BCC and a high expression in the other histopathological types; E-cadherin was absent in nodular, cystic and adenoid BCC and had a moderate expression in morpheaform BCC. In their study, proliferating cell nuclear antigen (PCNA) (proliferation-associated marker) was expressed in more than 60% of the epithelial tumor nuclei in morpheaform BCC, making it the most aggressive tumor of this anatomic area. Morpheaform and adenoid types also presented 20% expression of Ki67 labeling index, while the cystic type presented Ki67 expression in less than 10% of the malignant cells nuclei. CK8 was not expressed in all cases.

## Conclusions

Our study indicates that most of pBCC developed particularly in elderly female patients. Tumors were located in the lower eyelid with a left-side preference and most of them were histologically classified as nodular subtype. Most of our pBCCs were histologically classified as nodular subtype. pBCC IHC profile showed a strong immunopositivity for CK17, thus suggesting that the origin of pBCC is in the follicular germinative cells. We can conclude that our results showed a clinical, histological, and IHC profile which seems to be representative for Central and Eastern European countries, maybe due to the same genetic predisposition and environmental factors. However, there are some limitations in our study due to the reduced number of patients taken into consideration, but our series provides an overview of pBCC, as a basis of upcoming research on this issue, and could add essential information for ophthalmologists and pathologists dealing with this cancer.

### Conflict of interests

The authors declare that they have no conflict of interests.

### References

[1] Tesluk GC. Eyelid lesions: incidence and comparison of benign and malignant lesions. Ann Ophthalmol, 1985, 17(11):704–707.
[2] Lee SB, Saw SM, Au Eong KG, Chan TK, Lee HP. Incidence of eyelid cancers in Singapore from 1968 to 1995. Br J Ophthalmol, 1999, 83(5):595–597.
[3] da Cunha BN, de Souza DF, Marques N, Logan PT, Discepola J. Clinical and histopathological characteristics of periocular basal cell carcinoma in a low UV geographic region. Invest Ophthalmol Vis Sci, 2015, 56(7):3429.
[4] Jacob A. Observations respecting an ulcer of peculiar character, which attacks the eye-lids and other parts of the face. Dublin Hosp Rep Commun Med Surg, 1827, 4:232–239.
[5] Krompecher E. Der drüsenartige Oberflächenepithelkrebs (Ca. epitheliale adenoides). Zieglers Beitr Pathol Anatom, 1900, 28:1–41.
[6] Nékám L. Basalioma adenoides cysticum esete. Orv Hetil, 1901, 2:1–10.
[7] Krompecher E. Der Basalzellenkrebs. Gustav Fischer Verlag, Jena, 1903, 279.
[8] Rigel DS. Cutaneous ultraviolet exposure and its relationship to the development of skin cancer. J Am Acad Dermatol, 2008, 58(5 Suppl 2):S129–S132.
[9] Youssef KK, Van Keymeulen A, Lapouge G, Beck B, Michaux C, Achouri Y, Sotiropoulou PA, Blanpain C. Identification of the cell lineage at the origin of basal cell carcinoma. Nat Cell Biol, 2010, 12(3):299–305.

[10] Göppner D, Leverkus M. Basal cell carcinoma: from the molecular understanding of the pathogenesis to targeted therapy of progressive disease. J Skin Cancer, 2011, 2011: 650258.
[11] Saldanha G, Fletcher A, Slater DN. Basal cell carcinoma: a dermatopathological and molecular biological update. Br J Dermatol, 2003, 148(2):195–202.
[12] Wade TR, Ackerman AB. The many faces of basal-cell carcinoma. J Dermatol Surg Oncol, 1978, 4(1):23–28.
[13] Grostern RJ. Basal cell carcinoma. In: Older JJ. Eyelid tumors: clinical diagnosis and surgical treatment. 2nd edition, Manson Publishing, Ltd., 2003, 55.
[14] Wu A, Sun MT, Huilgol SC, Madge S, Selva D. Histological subtypes of periocular basal cell carcinoma. Clin Exp Ophthalmol, 2014, 42(7):603–607.
[15] Furdova A, Horkovicova K, Krcova I, Ondrusova M. Peri-ocular basal cell carcinoma. J Dermatol Clin Res, 2015, 3(4):1053.
[16] Florescu DE, Stepan AE, Mărgăritescu C, Ciurea RN, Stepan MD, Simionescu CE. The involvement of EGFR, HER2 and HER3 in the basal cell carcinomas aggressiveness. Rom J Morphol Embryol, 2018, 59(2):479–484.
[17] Hui YS, Kartiwa RA, Dwiwina RG. Characteristics of malignant eyelid basal cell carcinoma in Cicendo Eye Hospital Bandung from 2013 to 2015. Althea Med J, 2017, 4(1):148–151.
[18] Pornpanich K, Chindasub P. Eyelid tumors in Siriraj Hospital from 2000–2004. J Med Assoc Thai, 2005, 88(Suppl 9): S11–S14.
[19] Spraul CW, Ahr WM, Lang GK. [Clinical and histologic features of 141 primary basal cell carcinomas of the periocular region and their rate of recurrence after surgical excision]. Klin Monbl Augenheilkd, 2000, 217(4):207–214.
[20] Arlette JP, Carruthers A, Threlfall WJ, Warshawski LM. Basal cell carcinoma of the periocular region. J Cutan Med Surg, 1998, 2(4):205–208.
[21] Mallajosyula S. Surgical atlas of orbital diseases. 1st edition, Jaypee Brothers Medical Publishers, 2008, 252.
[22] Saldanha P, Shanthala PR, Upadhaya K. Cutaneous basal cell carcinoma: a morphological spectrum. Arch Med Health Sci, 2015, 3(1):24–28.
[23] Stanoszek LM, Wang GY, Harms PW. Histologic mimics of basal cell carcinoma. Arch Pathol Lab Med, 2017, 141(11): 1490–1502.
[24] Vantuchová Y, Čuřík R. Histological types of basal cell carcinoma. Scripta Medica (Brno), 2006, 79(5–6):261–270.
[25] LeBoit PE, Burg G, Weedon D, Sarasin A (eds). Pathology and genetics of skin tumours. 3rd edition, vol. 6, World Health Organization (WHO) Classification of Tumours, International Agency for Research on Cancer (IARC) Press, Lyon, 2006, 13–19.
[26] Hussain I, Soni M, Khan BS, Khan MD. Basal cell carcinoma presentation, histopathological features and correlation with clinical behaviour. Pak J Opthalmol, 2011, 27(1):3–7.
[27] Walling HW, Fosko SW, Geraminejad PA, Whitaker DC, Arpey CJ. Aggressive basal cell carcinoma: presentation, pathogenesis, and management. Cancer Metastasis Rev, 2004, 23(3–4):389–402.
[28] Rubin AI, Chen EH, Ratner D. Basal-cell carcinoma. N Engl J Med, 2005, 353(21):2262–2269.
[29] Bălăşoiu AT, Mănescu MR, Bălăşoiu M, Avrămoiu I, Pirici I, Burcea M, Mogoantă L, Mocanu CL. Histological and immuno-histochemical study of the eyelid basal cell carcinomas. Rom J Morphol Embryol, 2015, 56(2 Suppl):803–810.
[30] Cuevas-González MV, Vega-Memije ME, Cuevas-González JC, García-Vázquez FJ, Cháirez-Atienzo P, Ávila-Valdez R. Expression of CD34, Ki-67, p53 and cytokeratin AE1/AE3 in solid and adenoid basal cell carcinoma. Dermatol Rev Mex, 2016, 60(4):311–318.
[31] Ramezani M, Mohamadzaheri E, Khazaei S, Najafi F, Vaisi-Raygani A, Rahbar M, Sadeghi M. Comparison of EMA, CEA, CD10 and Bcl-2 biomarkers by immunohistochemistry in squamous cell carcinoma and basal cell carcinoma of the skin. Asian Pac J Cancer Prev, 2016, 17(3):1379–1383.
[32] Beer TW, Shepherd P, Theaker JM. Ber EP4 and epithelial membrane antigen aid distinction of basal cell, squamous cell and basosquamous carcinomas of the skin. Histopathology, 2000, 37(3):218–223.
[33] Anderson-Dockter H, Clark T, Iwamoto S, Lu M, Fiore D, Falanga JK, Falanga V. Diagnostic utility of cytokeratin 17 immunostaining in morpheaform basal cell carcinoma and for facilitating the detection of tumor cells at the surgical margins. Dermatol Surg, 2012, 38(8):1357–1366.
[34] Alessi E, Venegoni L, Fanoni D, Berti E. Cytokeratin profile in basal cell carcinoma. Am J Dermatopathol, 2008, 30(3): 249–255.

***Corresponding authors***

Mihaela Dana Turliuc, Associate Professor, MD, PhD, Department of Neurosurgery, Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, 16 University Street, 700115 Iaşi, Romania; Phone +40744–762 927, e-mail: turliuc_dana@yahoo.com

Anca Sava, Professor, MD, PhD, Department of Anatomy, Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, 16 University Street, 700115 Iaşi, Romania; Phone +40744–303 678, e-mail: dr.anca.sava.68@gmail.com

*Received: July 25, 2018*

*Accepted: April 14, 2019*