

# Clinicopathological Correlations in Cutaneous Squamous Cell Carcinomas

ALEXANDRA-ROXANA CIUCIULETE<sup>1</sup>, ALEX EMILIAN STEPAN<sup>2</sup>,  
BIANCA-CĂTĂLINA ANDREIANA<sup>2</sup>, CRISTIANA EUGENIA SIMIONESCU<sup>2</sup>

<sup>1</sup>PhD Student, Department of Pathology, University of Medicine and Pharmacy of Craiova, Romania

<sup>2</sup>Department of Pathology, University of Medicine and Pharmacy of Craiova, Romania

**ABSTRACT:** Squamous cell carcinomas (SCC) represent 20% of all nonmelanoma skin cancers, most tumors responding favorably to the conventional therapy. Incisional or excisional biopsy is essential for diagnosis and prognosis evaluation. The study included 103 cases of SCC, following the assessment of some clinical and histopathological aggressivity factors, which were digitally stored and statistically analyzed using comparison tests. The tumor grade was significantly associated with the histological variant, the maximum tumor size, the perineural and lymphovascular invasion, the depth of the invasion and the status of resection limits. The pT category was significantly associated with the location and maximum tumor size, perineural invasion, depth of invasion and status of resection limits. It was observed a significant association of tumor grade and pT category. The evaluation of the clinical and histological characteristics of SCC is an important step in obtaining relevant prognostic information and applying appropriate therapy.

**KEYWORDS:** Squamous cell carcinoma, clinical parameters, histopathological parameters.

## Introduction

Squamous cell carcinomas (SCC) represent about 20-30% of all nonmelanoma skin cancers [1-3]. Primitive SCC are often indolent tumors and with early and correct treatment they rarely develop metastases, with a 5-year survival rate of 90% [4].

There is a great diversity of histopathological varieties of these neoplasms, whose biological behavior varies from low metastatic potential to aggressive, rapidly invasive and metastatic potential [5,6]. Tumor excision and histopathological confirmation are necessary for all clinically suspected lesions, which will allow for the correct prognostic evaluation and treatment. Most nonmetastatic cutaneous SCC are cured after simple surgical excision, but approximately 4% of patients will show progression of neoplastic disease and will develop metastases [7]. As a result, the identification of these varieties is very important, early treatment of high-risk tumors leads to a reduction in the incidence of metastases and recurrences [8].

Although TNM staging is useful for estimating the evolution of the disease similar to other malignancies, there is a group of patients for whom, despite similar tumor characteristics, the individual risk cannot be estimated. In the present study we followed the statistical correlations between various clinical and pathological parameters of the cutaneous SCC.

## Material and Methods

This retrospective study included 103 cases of cutaneous SCC, diagnosed over a period of 1 year (2019-2020), from the Dermatology and Plastic Surgery Clinics of the Emergency County Hospital of Craiova and were processed in the Laboratory of Pathological Anatomy of the same hospital. The surgical excision specimens were fixed in buffered formalin 10%, processed using the paraffin embedding technique and Hematoxylin-Eosin (HE) staining. The classification of the lesions was done according to WHO (World Health Organization) and AJCC (American Joint Committee on Cancer) [9,10]. Study protocol was approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova.

We followed the assessment of clinical parameters (age, gender, maximum tumor size, topography) and histopathological parameters (histopathological type, tumor grade, depth of invasion, perineural and perivascular invasion, status of resection limits). Image acquisition was done with the Panthera L research microscope with 5 Mpixel digital camera (manufacturer MOTIC) and software integrated in the microscope. For statistical analysis we used the chi square comparison test ( $\chi^2$  test) in the SPSS10 software.

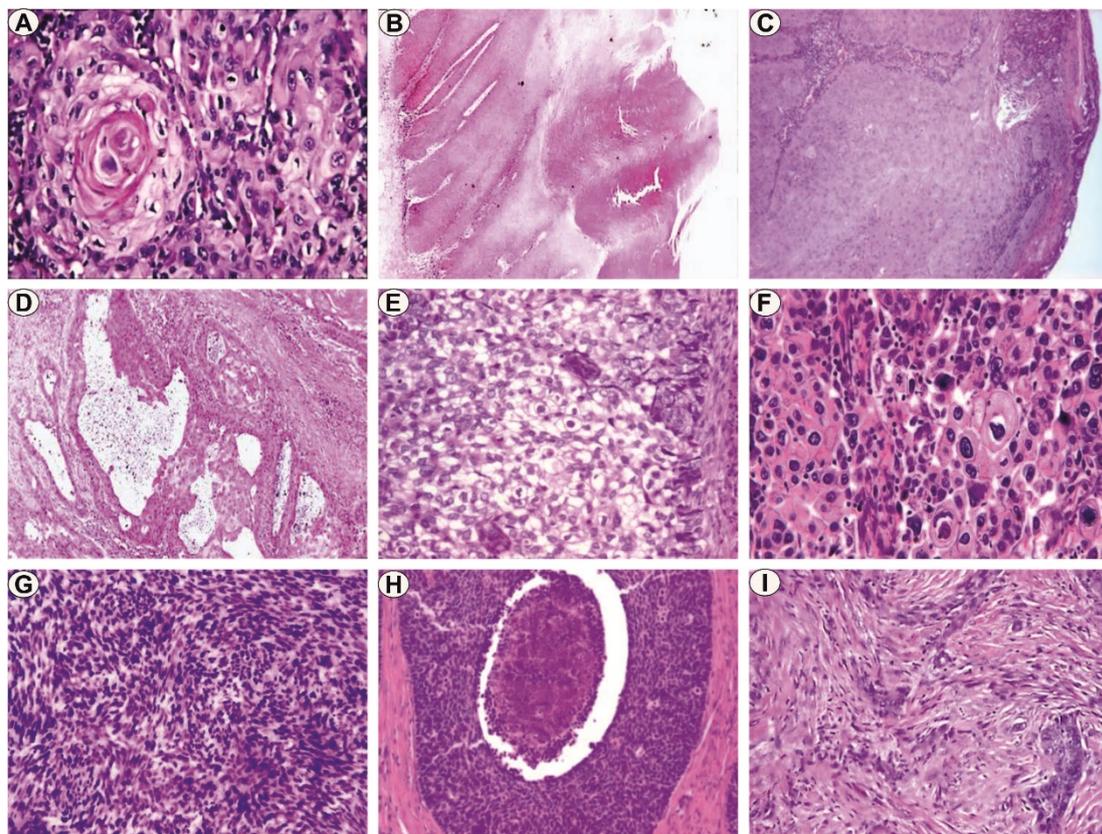
## Results

The study included 103 cases of cutaneous SCC diagnosed in patients with an average age of 74.2 years, predominantly in the seventh and

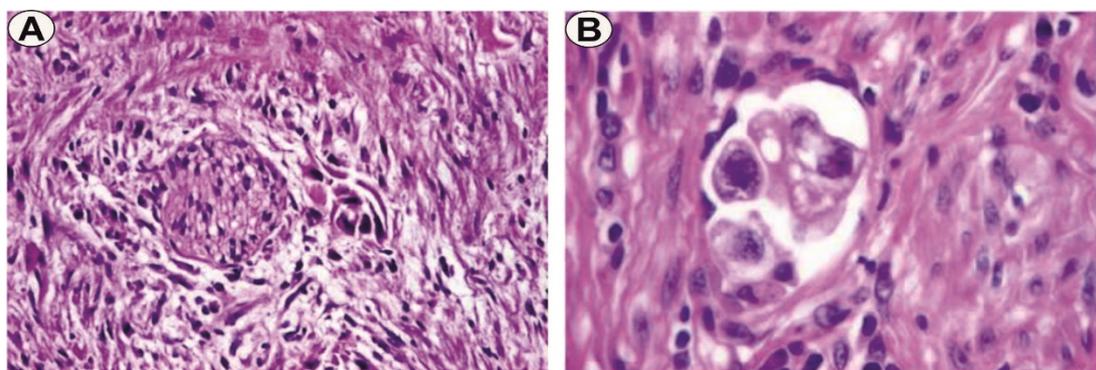
eighth decades of life (30.1% and 35.9%, respectively), especially in males (59.2%) (Table 1). The most common location was the head and neck (76.7%), followed by the upper limbs (9.7%), lower limbs (6.8%), groin (4.9%) and thorax (1.9%). When categorized by maximum tumor size, most tumors were part of the below 20mm category (60.2%), followed by those between 20-40mm (23.3%) and over 40mm (16.5%).

Histopathological evaluation of tumor grade indicated the highest incidence for well-differentiated tumors (G1) (49.5%),

followed by moderately-differentiated SCC (G2) (36.9%) and poorly differentiated (G3) (13.6%). Each tumor grade included several growth patterns, SCC G1 being mostly represented by the conventional (41.7%) and verrucous types (7.7%) (Figure 1A-B), SCC G2 also included the most frequent conventional aspects (25.2%) but also the acantholytic type (9.7%) and clear cells type (1.9%) (Figure 1C-E), and SCC G3 corresponded more frequently to the conventional type (8.7%), as well as basaloid (1.9%), spindle cell (1.9%) and desmoplastic types (1%) (Figure 1F-I).



**Figure 1.** SCC, HE staining. **A.** Keratinized SCC, x200; **B.** Verrucous SCC, x40; **C.** Nonkeratinized SCC, x40; **D.** Acantholytic SCC, x40; **E.** SCC with clear cells, x100; **F.** Nonkeratinized SCC, x200; **G.** SCC with fusiform cells, x100; **H.** Basaloid SCC, x100; **I.** Desmoplastic SCC, x100.



**Figure 2.** **A.** Perineural invasion, desmoplastic SCC, HE staining, x100; **B.** Vascular invasion, non-keratinized SCC, HE staining, x200.

Perineural invasion was identified in 9.7% of cases, and lymphovascular invasion in 3.9% of cases (Figure 2A-B). The assessment of the depth of the tumor invasion indicated that frequently the tumors were invasive in the deep dermis (43.6%) and hypodermis (30.2%), the rest of the cases having invasion in the deeper structures (muscles, cartilage and bones, constituting 20.4%, 3.9% and 1.9% respectively) (Table 1). The analysis of the invasion of the surgical resection limits showed the invasion in almost a quarter of the investigated cases (24.3%). The classification of SCC by pT category indicated that the most cases

corresponded to the subcategory pT1 (46.6%), followed by the subcategories pT3 (33%), pT2 (17.5%) and pT4 (1.9%) (Table 1).

Statistical analysis of the investigated clinical-histopathological parameters in relation to the tumor grade of the tumors and the pT category indicated a series of significant associations (Table 1). The tumor grade was significantly associated with the histological variant, the maximum tumor size, the perineural and lymphovascular invasion, the depth of the invasion, the status of the resection limits and the pT category.

**Table 1. Cases distribution and associations of the investigated clinico-pathological parameters.**

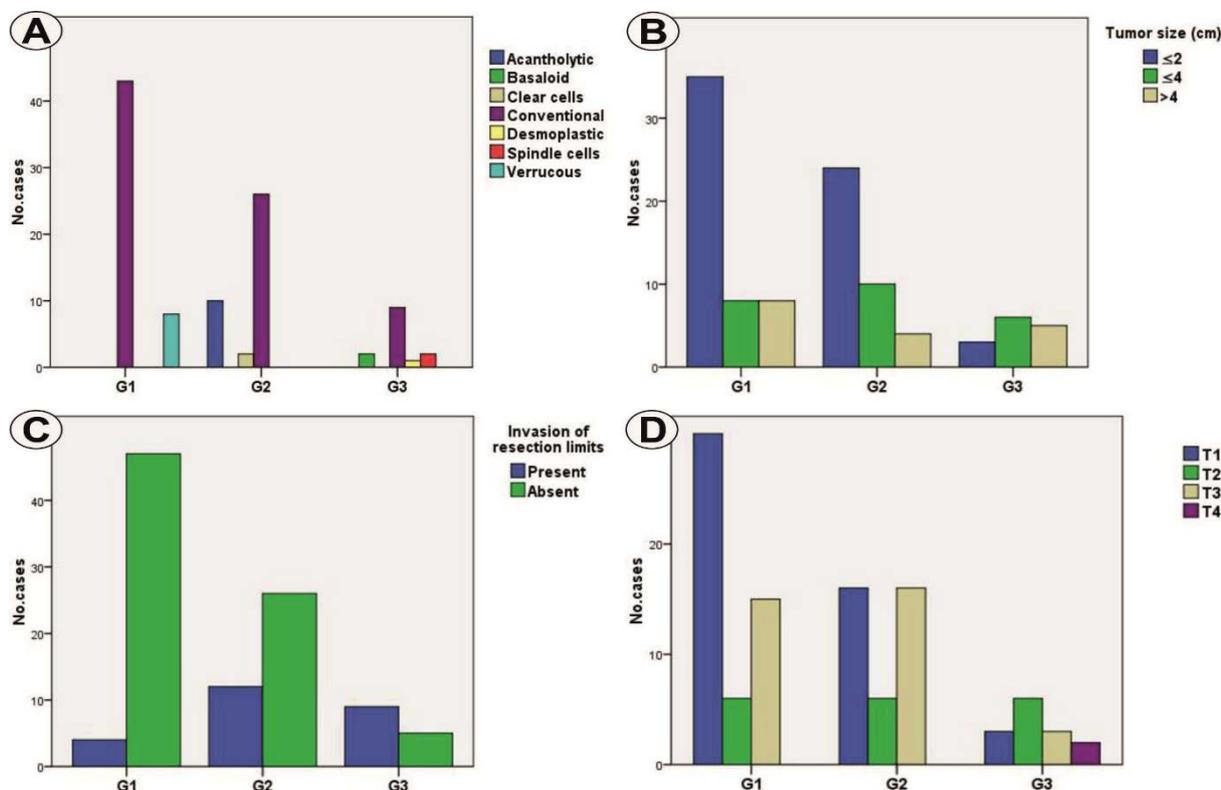
Parameters		No. cases	Percentages %	p value ( $\chi^2$ test)	
				Tumor grade	pT
<b>Age</b>	≤60	15	14.6	p>0.05	p>0.05
	>60	88	85.4		
<b>Gender</b>	female	42	40.8	p>0.05	p>0.05
	male	61	59.2		
<b>Localization</b>	head and neck	79	76.7	p>0.05	p=0.004
	thorax	2	1.9		
	upper limb	10	9.7		
	lower limb	7	6.8		
	groin region	5	4.9		
<b>Histopathological type</b>	conventional	78	75.8	p=0.001	p>0.05
	verrucous	8	7.8		
	acantholytic	10	9.7		
	with clear cells	2	1.9		
	basaloid	2	1.9		
	with spindle cells	2	1.9		
	desmoplastic	1	1		
<b>Maximum size (mm)</b>	≤20	62	60.2	p=0.019	p=0.000
	>20-<40	24	23.3		
	≥40	17	16.5		
<b>Tumor grade</b>	G1	51	49.5	-	p=0.000
	G2	38	36.9		
	G3	14	13.6		
<b>Perineural invasion</b>	-	10	9.7	p=0.003	p=0.001
<b>Lymphovascular invasion</b>	-	4	3.9	p=0.043	p=0.003
<b>Depth of invasion</b>	dermis	45	43.6	p=0.000	p=0.000
	hypoderm	31	30.2		
	muscle	21	20.4		
	cartilage	4	3.9		
	bones	2	1.9		
<b>Positive resection limits</b>		25	24.3	p=0.001	p=0.001
<b>pT category</b>	pT1	49	47.6	p=0.000	-
	pT2	18	17.5		
	pT3	34	33		
	pT4	2	1.9		

The pT category was significantly associated with the localization and maximum tumor size, the tumor grade, perineural and lymphovascular invasion, depth of invasion and status of resection limits. We observed statistically significant associations of the histological type with tumor grade, the spindle cell SCC, basaloid SCC and desmoplastic SCC being associated with high grade (G3) ( $p < 0.001$ ,  $\chi^2$  test), but not with the pT category ( $p > 0.05$ ,  $\chi^2$  test) (Figure 3A).

Tumor localization was not associated with tumor grade ( $p > 0.05$ ,  $\chi^2$  test), in contrast to the pT category which was significantly associated with neoplasia localization ( $p = 0.004$ ,  $\chi^2$  test). Statistically significant associations were noted between tumor size and tumor grade, G1/G2 tumors predominating in tumors with dimensions  $\leq 2$ cm, and G3 tumors predominated

in tumors with dimensions  $\geq 2$ cm ( $p = 0.019$ ,  $\chi^2$  test) (Figure 3B). Regarding the pT category, we found that the size of the tumors was higher in the advanced pT categories (pT3 and pT4), showing a statistically significant association ( $p < 0.001$ ,  $\chi^2$  test).

Regarding the resection limits, the invaded ones predominated in the cases of SCC G3, an association that was statistically significant ( $p = 0.001$ ,  $\chi^2$  test) (Figure 3C). Also, the interest of resection limits was significantly associated with pT, the invaded limits being frequently present in the cases of pT3 and pT4 categories ( $p < 0.001$ ,  $\chi^2$  test). Tumor grade presented significant associations with the depth of invasion, in G3 cases predominated the invasion of the hypodermis, followed in the order of frequency of dermis, bone and cartilage ( $p < 0.000$ ,  $\chi^2$  test).



**Figure 3. Cases distribution depending on tumor grade and histological type (A), tumor size (B), the status of resection limits (C) and pT category (D).**

The depth of invasion was significantly associated with pT, the invasion in bone tissue and muscle predominating in the categories pT3 and pT4 ( $p = 0.000$ ,  $\chi^2$  test). Perineural invasion was present in tumors G2 and G3, a statistically significant association ( $p = 0.003$ ,  $\chi^2$  test), as well as in cases of advanced pT categories (pT3 and pT4) ( $p = 0.001$ ,  $\chi^2$  test).

Lymphovascular invasion was statistically significantly associated with moderate SCC, respectively poorly differentiated (G2/G3) ( $p = 0.043$ ,  $\chi^2$  test), as well as with SCC included in the subcategory pT4 ( $p = 0.003$ ,  $\chi^2$  test). In addition, we observed statistically significant associations between G2/G3 tumors and advanced pT category (pT3 and pT4) ( $p < 0.001$ ,  $\chi^2$  test) (Figure 3D).

## Discussions

SCC staging uses several clinico-pathological parameters that indicate the aggressive potential of tumors, such as tumor size, depth of invasion, perineural and lymphatic/vascular invasion, and histological differentiation of the tumor, although the last one was not included in the most recent AJCC classification [10].

High tumor grade, perineural invasion, Breslow index more than 2mm, diameter more than 20mm, and temple location were associated with an increased risk of recurrence and metastasis [3].

On the other hand, when patients have 2 or more parameters associated with increased risk, about 20% of them will also have metastases in the sentinel nodes [11].

The average age reported at the time of diagnosis is 78 years, of which 82.6% are over 70 years old [12].

In our study, tumor grade and pT category did not indicate statistically significant associations with the age and gender of the patients.

Regarding the localization of neoplasms, other authors have also reported tumors located in the head and neck area for about 80% of patients, 20% being distributed fairly evenly over the rest of the body skin [12].

In our study, there were significant correlations with the pT category, but not with tumor grade.

Tumor size is also a determinant factor of tumor recurrence and metastasis [1].

SCC with dimensions less than 2cm rarely metastasize and have a low risk of recurrence, unlike SCC with dimensions over 2cm which have a significant risk of metastasis and recurrence [1,13].

Tumor diameter over 2cm doubles the risk of recurrence and triples the rate of metastases [14].

In our study we noticed statistically significant associations between tumor size and tumor grade.

Also, the size of the tumors was higher in the advanced pT categories.

However, tumor size is only a prognostic criterion, and other histopathological criteria for tumors are needed, such as the depth of invasion, the presence of perineural and lymphovascular invasion, and tumor grade, because many T1 and T2 patients may develop regional and distal metastases [15].

Thus, tumor grade is another factor used in developing the prognosis [16], although it is

evaluated semi-quantitatively and as a result the classification of tumors is quite subjective [17].

In a large study, the tumor grade was an independent prognostic factor for metastatic disease and overall survival or survival without metastasis at 5 years, being significantly higher in well-differentiated SCC (70%) compared to moderately and poorly differentiated (51%) [17].

In another large study, which included 1832 tumors, overall mortality was associated with high grade [7].

Similarly, other authors have found that poorly differentiated or undifferentiated SCC are more prone to the development of lymph node metastases than moderate or well-differentiated SCC [18].

The histopathological subtypes with the highest risk of metastasis are desmoplastic and acantholytic variants, with a metastasis rate between 21.4 and 44.4% [6,19].

In addition, the desmoplastic growth pattern is an independent risk factor for local recurrence, with 10 and 6 times higher risk of local recurrence and metastasis than other types of SCC [20].

For the evaluated case studies, we found significant associations between moderate and poorly differentiated SCC and the advanced pT category.

Other essential components of the evaluation of the malignant potential of a tumor is the presence of perineural and perivascular invasion [21,22].

Perineural invasion is identified in approximately 5-10% of patients with cutaneous SCC, disease-specific survival at 3 years being 64% in patients with perineural invasion, compared to 91% in those without perineural invasion [23].

In a large study, the authors reported the association of perineural invasion with disease-specific mortality [7].

Other studies indicate perineural invasion as a marker of unfavorable prognosis and an independent increased risk factor for desmoplastic SCC [24] and spindle cell SCC [16].

In this study, perineural invasion was present in SCC G2 and G3, classified in the advanced pT categories.

Moore BA et al. suggested that lymphovascular invasion increases the risk of metastasis in SCC, being an independent predictor of lymph node metastases, 40% of patients with metastases associating

lymphovascular invasion, compared with only 8% of those without metastases [25].

In addition, recurrent SCC are associated with a higher rate of perineural invasion (24% vs. 10%), lymphovascular invasion (17% vs. 8%) and subcutaneous tissue (30% vs. 10%) [23].

In this study lymphovascular invasion showed statistically significant associations with SCC G2/G3 and advanced pT (pT4).

The depth of invasion is also a determinant factor of tumor recurrence and metastasis [1].

Deep invasion is defined as invasion beyond subcutaneous fat or >6mm (measured from the granular layer to the base of the tumor) [10].

Branscht KD et al. evaluated several variables and concluded that SCC with dimensions less than 2mm were not associated with the risk of recurrence or metastasis, a risk that increased to 4% for SCC with dimensions between 2.1-6.0mm and at 16% for SCC greater than 6mm [20].

Veness et al. did not report metastases in tumors less than 2mm thick, these being present in 17% of tumors between 2 and 4mm thick and in 83% of tumors greater than 4mm [26].

Depth of invasion over 2mm associated ten times higher risk of local recurrence, and beyond subcutaneous fat increases the risk of metastasis eleven times [14].

In our study the depth of invasion showed significant associations with tumor grade and pT category, in SCC G3 pT3/T4 tumors predominating the invasion in dermis, hypodermis, bone tissue and cartilage.

Incomplete excision of tumors is another predictor of unfavorable prognosis in cutaneous SCC.

In a retrospective study, which included over 200 articles, the authors found incomplete excision rates ranging from 0.4 to 35.7% [27].

The recommended surgical excision limits in Europe are 5mm for low-risk tumors and 10mm for high-risk tumors and deep invasion to the hypodermis [28].

In our study, the invasion of resection limits predominated in SCC G3, these being frequently present in the cases of pT3 and pT4.

Recent studies recommend for high-risk SCC excision with a peripheral limit of more than 6mm and the deep limit extended at least to the next anatomical plane [29].

## Conclusions

The study indicated statistically significant correlations of the analyzed clinical and histopathological parameters.

Identifying aggression factors in relation to the prognosis of SCC can be useful for developing better prevention and therapy strategies.

## Conflict of interests

None to declare.

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**Corresponding Author: Alex Emilian Stepan, Department of Pathology,  
University of Medicine and Pharmacy of Craiova, 66 1 May Avenue, 200628 Craiova,  
e-mail: astepan76@yahoo.com**

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