JBUON 2021; 26(3): 1148-1158
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

ORIGINAL ARTICLE

Tracking the risk factors associated with high-risk cSCC: A 10-year, Two-Institution, Greek study

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*This author received scholarship from Onassis Foundation.

Summary

Purpose: *We sought to identify independent risk factors for positive sentinel lymph node biopsy (SLNB), local recurrence (LR), metastasis (M) and death caused by cutaneous squamous cell carcinoma (cSCC) (DCS) in high-risk cSCC patients. Moreover, we compared the Brigham and Women's Hospital (BWH) system with the previous used in Greece (based on tumor size) and proposed a new classification system.*

Methods: *1,524 cSCC patients were enrolled between January 2004 and December 2014, from two medical institutions. Potential risk factors for SLNB (local recurrence/LR, metastasis/M, death caused by SCC/DCS) were analyzed by univariate and multivariate Cox logistic regression models.*

Results: *Of the included patients with a median follow-up of 60 months 107 developed local recurrence (7%) while 84 developed metastases (5.5%). Among 36 patients undergoing sentinel lymph node biopsy (SLNB), 25% showed a positive SLNB with a false-negative result (11%). On multivariate analysis, key prognostic factors for LR were tumor diameter ≥2 cm, poor differentiation, incomplete excision and perineural invasion and for M were high-risk tumor site, tumor diameter ≥2 cm, poor differentiation, invasion beyond subcutaneous tissue, incomplete excision, perineural invasion and recurrence. DCS seems to be affected by tumor diameter ≥ 2 cm, poor differentiation, invasion beyond subcutaneous tissue, incomplete excision, perineural invasion and recurrence independently.*

Conclusions: *These suggest the determined role of tumor diameter of cSCCs. Harnessing knowledge and collecting the up-to-date data along the clinical journey of high-risk cSCC, the future looks bright (development of new clinical trials, adjuvant therapies and tumor staging with SLNB).*

Key words: *cutaneous squamous cell carcinoma, cSCC, high-risk cSCC, prognosis, sentinel lymph node biopsy, staging criteria*

Introduction

Cutaneous squamous cell carcinoma (cSCC) originates from keratinizing cells of the epidermis or its appendages. According to existing data, the higher incidence is evident in USA with 356/100.000 person-years and in Australia with 400/100.000 person-years, while in Europe a much

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Received: 07/11/2020; Accepted: 11/12/2020

lower incidence is registered (16/100.000 person-years). In particular, the higher rates in Europe concern the southern European countries (Spain, Italy, and Portugal). Unfortunately, in Greece there is no official national registration of non-melanoma skin cancer cases [1,2].

The majority of cSCCs is low-risk, with a 90% 5-year survival rate after surgical excision. Low-risk patients are unlikely to have local recurrence (10%) or lymph node metastasis (5%) [1,3]. Nevertheless, there is a subgroup of cSCC with an increased risk of local recurrence (10-47.2%) and metastatic potential (15-38%) [4], which are characterized as high-risk.

In Greece, clinicians used to characterize cSCC unofficially as high-risk based on the tumor size (≥ 2 cm) and started in 2004 to perform sentinel lymph node biopsy (SLNB). The National Comprehensive Cancer Network (NCCN), the AJCC and the Brigham and Women's Hospital tumor stage (BMW) suggest distinct criteria based on specific high-risk factors with no sufficient evidence on the improvement of prognosis of these patients [5].

The primary endpoint of the present study of cSCC was designed to evaluate certain clinical-pathological features of cSCCs with local recurrence, metastasis, sentinel lymph node (SLN) involvement as well as overall survival (OS).

The secondary endpoint was to compare an existing staging system (BWH) with the previous system used in Greece and a modified classification system suggested by our team, which will be described thoroughly below.

Methods

Study population

This retrospective study included patients with histologically proven primary invasive cSCC, treated between January 1, 2004 and December 31, 2014 at two Greek tertiary care centers (Andreas Syggros Hospital of Cutaneous and Venereal Diseases, School of Medicine, University of Athens and Alexandroupolis University Hospital, School of Medicine, Democritus University of Thrace).

Patient medical and demographic data were collected from databases of the departments of plastic surgery while the histopathologic results were obtained from electronic medical files.

This study was approved by the local Ethics committee and was conducted in accordance with the Guidelines for Good Clinical Practice (GCP) and in compliance with the Declaration of Helsinki. Data was anonymized prior to analysis with respect to data protection regulations. Our multidisciplinary team consisted of dermatologists, plastic surgeons, radiologists, pathologists, oncologists, and primary care nurses.

Exclusion criteria included the following: patients with non-cutaneous (C) SCC (mucosal, anogenital), *in situ* cSCC and recurrent (nonprimary) SCC. Furthermore, patients with missing clinical data concerning medical history or with poor follow-up (<1 visit) were not included. Patients with multiple invasive cSCCs were enrolled in the study, based on the timeline of their first lesion to achieve the longest follow-up. In cases with multiple simultaneously invasive cSCCs appearing, the most aggressive lesion was taken into account, based on the high-risk criteria of the pathological report. These assumptions were obligatory because we wanted to assign one tumor per patient. Multiple cSCCs from each patient may function as biased data, especially in terms of demographic factors (age, gender, immune status). So, if a patient had simultaneously both high- and low-stage tumors in their first visit, high-stage tumors were selected. These tumors are rarer and contribute more than low-risk to patient's prognosis.

Outcomes of interest, including local recurrence, metastasis, sentinel lymph node involvement and death related to cSCC, were recorded. Local recurrence was considered if the pathology report documented development of invasive cSCC at the same site as primary tumor. Recurrence-free survival (RFS) was considered from the date of first excision until relapse. Metastasis was defined as pathologically confirmed cSCC occurring in a draining nodal basin of the primary cSCC and/or distant organs or surrounding tissue, such as bone, with no other potential source. In many cases distant metastasis was determined radiologically (CT, MRI, PET Scan). Metastasis-free survival (MFS) was determined from the date of first excision until metastasis. SLNB was used to detect micrometastases from high-risk cSCC in cases with clinically negative nodes. Death caused by cSCC (DCS) was defined as directly resulting from the disease or its direct complications. The baseline for follow-up documentation was defined as the operation date. OS represented the time between surgical treatment of the tumor and either death or last follow-up. Patients alive at the time of last follow-up were recorded as censored data in terms of survival analysis. Follow-up visits included clinical and lymph node ultrasound examinations every 3 months for the first 2 years, every 6 months for another 3 years and annually thereafter. When patients could not attend our institution, follow-up telephone appointments were used for follow-up [2,6].

Additional patient's demographic information included age at diagnosis of the primary tumor, gender, history of radiation treatment, previous burns, or other scarring processes in the area of the tumor and the immunocompetency. Tumors were assigned to one of the following three anatomic regions: head and neck, trunk and extremities. Tumors developed in lips, ear and periauricular areas, soles, burn sites or actinic dermatitis areas were characterized as high-risk tumor sites. Furthermore, histopathologic features of the primary tumor included: anatomic location, grade of differentiation (poor vs moderate/well), depth of invasion, subtype of cSCC, perineural or lymphovascular

invasion, tumor thickness and complete tumor excision [7]. Breslow thickness was not included in most pathology reports. Instead, anatomical depth of invasion was recorded (dermis, subcutaneous fat, muscle). Tumor diameter in many cases was obtained from the largest measurement of the gross excisional specimen. Finally, treatment of the metastatic tumor including radiotherapy, chemotherapy, or lymph node dissection, alone or combination of them, were also included.

Tumor staging

BWH tumor staging system was assigned to all tumors of this study for better prognostic discrimination. In BWH staging, tumors are classified based on the presence of 4 risk factors: poor differentiation, a diameter of 2 cm or greater, perineural involvement and invasion beyond the subcutaneous fat. Tumors were high-risk if they were classified as BWH T2b or T3 and low risk if T1 or T2a. The latter have a reportedly small risk of local risk, metastasis, and death [8]. Nowadays an alternative tumor staging inspired by NCCN and BWH guidelines is recommended by our team based on 2 or more risk factors for high-risk tumor characterization. These factors comprise high-risk tumor location (ear, lip, inflammation site), size ≥ 2 cm, poor differentiation, invasion beyond subcutaneous fat, immune status, invasion or lymphovascular involvement, cSCC subtype and incomplete excision.

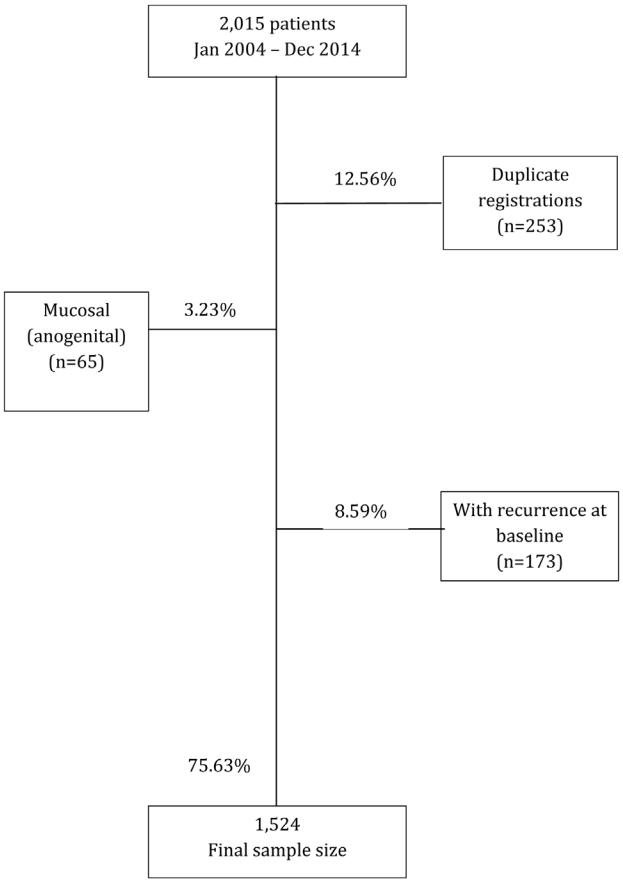

Figure 1. Flowchart of patient inclusion.

Table 1. Demographic and clinical characteristics of patients with cSCC for the period 2004-2014

Variables	*Total (n=1.524)*
Age (years), mean (SD)	74.86 (11.44)
Gender, n (%)	
Men	1038 (68.11)
Women	486 (31.89)
Period of visit, n (%)	
2004 - 2009	752 (49.34)
2010 - 2014	772 (50.66)
Tumor localization, n (%)	
Extremities	209 (13.71)
Head & Neck	1269 (83.27)
Trunk	46 (3.02)
Tumor site, n (%)	
High-risk	536 (35.17)
Low-risk	988 (64.83)
High-risk tumor site, n (%)	
Lip	390 (72.76)
Auricle / periauricular area	131 (24.44)
Sole	13 (2.43)
Chronic inflammation sites (burn/ actinic dermatitis)	2 (0.38)
Follow-up (months), mean (SD)	47.47 (21.11)
Local recurrence, n (%)	
No	1417 (92.98)
Yes	107 (7.02)
Relapse-free survival (months), median (25th-75th)	9 (4-34)
Number of relapses, n (%)	
0	1417 (92.98)
1	89 (5.84)
2	14 (0.92)
3	4 (0.26)
Overall metastasis, n (%)	
No	1440 (94.49)
Yes	84 (5.51)
Metastasis diagnosed at primary tumor excision, n (%)	
No	1509 (99.02)
Yes	15 (0.98)
Type of metastasis I, n (%)	
Distant	7 (10.29)
Nodal	57 (83.82)
Distant and nodal	4 (5.88)
Type of metastasis II, n (%)	
Distant	4 (4.76)
Distant and surrounding tissue	3 (3.57)
Surrounding tissue	16 (19.05)
Nodal	55 (65.48)
Nodal and distant	4 (4.76)
Nodal and surrounding tissue	2 (2.38)

Continued on the next page

Variables	*Total (n=1.524)*
Metastasis-free survival (months), median (25th-75th) (n=69)	7 (4-12)
All-cause-death, n (%)	
No	1.435 (94.16)
Yes	89 (5.84)
Cause of death, n (%)	
cSCC	60 (67.42)
Other	29 (32.58)
All-cause-death survival (years), median (25th-75th)	1.97 (1.19-4.51)
Disease-specific survival (years), median (25th-75th)	1.91 (1.15-3.63)

Statistics

All data were collected via Microsoft Excel, and statistical analyses were performed using Stata version 13.0 (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP). Descriptive analysis assessed patients' and cSCC histological characteristics. Cox proportional hazards regression models were used to identify predictors of development of local recurrence, metastasis and death due to SCC. Local relapse-free survival, metastasis-free survival, death caused by SSCC-survival and overall survival of low and high-risk patients based on BWH staging system were estimated by Kaplan-Meier method and log-rank test. Univariate and multivariate hazard ratios were calculated with 95% confidence intervals (95% CI). Variables included in the multivariate model were all those with $p<0.25$ in the univariate analyses as well as age and sex as potential confounders. We further explored which factors were associated with the presence of positive SLNB. Crude and adjusted odds ratios (OR) of positive SLNB along with 95% CI were obtained using logistic regression. All statistical tests were performed using a 2-sided 5% type I error rate.

Results

Patient characteristics

The database involved 2,120 consecutive patients with primary invasive (non-*in situ*) cSCC. After medical record review, 105 patients were excluded because of insufficient history, tumor's pathology report or non-existent follow-up, leaving 2015 patients in the study. Of these patients, 173 were ineligible because they presented with recurrent tumors and 65 were excluded due to anogenital SCC. So, from the rest of 1.777 patients, 253 were also excluded because of their registration as duplicate entry due to multiple cSCCs (Figure 1). The final pool of patients was 1.524 and the median (25th-75th) follow-up period was 60 (31-60) months. The mean age (standard deviation) of patients at primary surgical excision was 74.9 (11.4) years. There were 1.038 male and 486 female patients (Table 1). A total of 19 patients (1.25%) were immunosuppressed for a variety of reasons, including organ transplantation (n=9), autoimmune diseases under immunosuppressive drugs (n=5), chronic lymphocytic leukemia (n=4) and HIV immunosuppression (n=3) (Table 2).

Table 2. High-risk tumor or patient features, 2004-2014

Variables	*Total (n=1.524)*
Immunosuppresion, n (%)	
No	1505 (98.75)
Yes	19 (1.25)
Type of immunosuppresion, n (%)	
HIV	3 (15.79)
Drug-induced	5 (26.32)
Leukemia	2 (10.53)
Transplantation	9 (47.37)
Max tumor diameter (cm), median (25^{th}-75^{th})	1.80 (1.00-2.70)
Max tumor diameter, n (%)	
High-risk (≥2 cm)	674 (44.23)
Low-risk (<2 cm)	850 (55.77)
Subtype of cSCC, n (%)	
No	1.510 (99.08)
Yes	14 (0.92)
Invasion beyond subcutaneous tissue, n (%)	
No	1.423 (93.37)
Yes	101 (6.63)
Tumor thickness, median (25th-75th)	0.50 (0.30-0.70)
Tumor thickness, n (%)	
<0.6 cm	42 (61.76)
≥0.6 cm	26 (38.24)
Complete excision, n (%)	
Yes	1.424 (93.44)
No	100 (6.56)
Ulceration, n (%)	
No	583 (38.12)
Yes	943 (61.88)
Perineural invasion, n (%)	
No	1.471 (96.52)
Yes	53 (3.48)
Lymphovascular invasion, n (%)	
No	1.482 (97.24)
Yes	42 (2.76)
Associated inflammatory infiltrate, n (%)	
No	1.427 (93.64)
Yes	97 (6.36)
Differentiation, n (%)	
Poor	282 (18.50)
Moderate/well	1.242 (81.50)

Table 3. Characteristics of management of patients with cSCC, 2004-2014

Variables	*Total (N=1,524)*
Sentinel lymph node biopsy (SLNB), n (%)	
No	1488 (97.64)
Yes	36 (2.36)
Result of SLNB, n (%)	
Negative (-)	27 (75.00)
Positive (+)	9 (25.00)
Number of SLNs, median (25th-75th)	1 (1.00-1.00)
Max diameter of SLNs, median (25th-75th)	2.20 (2.00-2.70)
Area of SLNB, n (%)	
Axillary area	2 (5.56)
Cervical area	25 (69.44)
Inguinal area	9 (25.00)
Treatment of metastasis, n (%)	
Conservative	27 (46.55)
Surgical	31 (53.45)
Treatment options of metastasis, n (%)	
Radiotherapy	12 (20.69)
Complete lymph node dissection	12 (20.69)
Chemotherapy	2 (3.45)
Combination of treatments	32 (55.17)
Result of complete lymph node dissection (CLND), n (%)	
Negative (-)	6 (23.08)
Positive (+)	20 (76.92)
Number of lymph nodes in CLND, median (25th-75th)	8 (6-14)
Max diameter of lymph nodes (LN) in CLND (cm), mean (SD)	1.45 (0.54)
Percentage of infiltrated LN in CLND, median (25th-75th)	41.43 (25.00 - 80.00)
Area of CLND, n (%)	
Axillary area	3 (9.68)
Cervical area	24 (77.42)
Inguinal area	4 (12.90)
Number of parotidectomies, n (%)	
Superficial	15 (88.24)
Preventive	2 (11.76)
Result of parotidectomies, n (%)	
Negative (-)	6 (35.29)
Positive (+)	11 (64.71)

Table 4. Classification of patients according to staging systems, 2004 - 2014

Variables	*Total (n=1,524)*
Number of BWH factors, n (%)	
0	712 (46.72)
1	580 (38.06)
2	172 (11.29)
3	47 (3.08)
4	13 (0.85)
BWH classification, n (%)	
T1	712 (46.72)
T2a	580 (38.06)
T2b	219 (14.37)
T3	13 (0.85)
BWH classification (Low/High-risk), n (%)	
Low-risk (< 2 factors)	1.292 (84.78)
High-risk (≥ 2 factors)	232 (15.22)
Our classification system, n (%)	
0	244 (16.01)
1	504 (33.07)
2	492 (32.28)
3	195 (12.80)
4	68 (4.46)
5	17 (1.12)
6	3 (0.20)
7	1 (0.07)
Our new classification system, n (%)	
Low-risk (< 2 factors)	748 (49.08)
High-risk (≥ 2 factors)	776 (50.92)

Lesion characteristics at presentation

Of the 1,524 index-lesions documented, 1,269 occurred in the head and neck, 209 in the extremities and 46 in the trunk. There were 536 high-risk cases of which 390 were distributed within the lip area (72.76%), 131 in the ear/periauricular area (24.44%), 13 in the sole (2.43%) and lastly, 2 in chronic inflammatory sites (0.38%) (Table 1). The median diameter of cSCCs at presentation was 1.80 cm (1.00-2.70) and categorized in two groups (cut-off 2 cm diameter): 674 cases (44.23%) were ≥ 2 cm and 850 (55.77%) < 2 cm. Tumor thickness was available only for 68 tumors with a median 0.50 cm (0.30-0.70). Moreover, 101/1,524 cSCCs invaded beyond the subcutaneous tissue plane; 282 (18.50%) tumors were poorly differentiated while 1.242 (81.50%) moderately/well. Of the 1.524 tumors, 53 featured perineural involvement and 42 lympho-vascular infiltration. Furthermore, 943 (61.88%) presented ulceration, 97 (6.36%) with mild to moderate chronic inflammatory cell infiltrate and 14 (0.92%) were characterized by cSCC subtypes. In total, all the primary tumors were treated with surgical excision. 1.424 (93.44%) were excised completely and 100 (6.56%) had positive excision margins (Table 2).

Sentinel lymph node biopsy

Thirty-six patients with tumors located in the abdomen, lip, upper arm, and knee under-

Table 5. Univariate and multivariate logistic regression model for examining the possible factors of positive SLNB in 36 patients* with cSCC, period 2004-2014

Variables	*Univariate analysis Crude OR (95% CI)*	*Multivariate analysis Adjusted OR (95% CI)*
Age (years)	1.03 (0.97, 1.10)	0.98 (0.90, 1.06)
Gender (Women/Men)	10.00 (1.42, 70.30)	10.70 (0.76, 150.28)
Tumor site (High/Low-risk)	8.80 (1.62, 47.80)	
Max tumor diameter (≥2/< 2 cm)	11.64 (1.27, 106.72)	9.58 (0.88, 103.85)
Differentiation (poor/ moderate-well)	1.43 (0.28, 7.30)	
Invasion beyond subcutaneous tissue (Yes/ No)	1.00 (0.09, 11.03)	
Complete excision (No/ Yes)	1.56 (0.13, 19.60)	
Perineural invasion (Yes/ No)	1.64 (0.25, 10.95)	

OR: odds ratio; CI: confidence interval. *None of these 36 patients was immunosuppresed. Also, subtype of cSCC, ulceration, lymphovascular invasion and associated inflammatory infiltrate were not described in this group of patients.

went SLNB. Nine out 36 patients (25%) had positive SLNB with median one excised lymph node and size 2.20 cm (Table 3). None of these patients was immunocompromised and none of these tumors showed the following features: ulceration, inflammatory cell infiltration, lymphovascular involvement and cSCC subtype. Univariate analysis revealed high-risk tumor location and tumor diameter ≥ 2 cm as the only parameters significantly predicting a positive SLNB. Multivariate analysis that followed revealed that tumor diameter ≥2 cm was the only independent risk factor in our cohort for positive SLNB, with female sex reaching the limit of statistical significance (p=0.06) (Table 5).

Local recurrence

Confirmed recurrence data was available for 107 patients (7.02%) after a median (25th-75th) period of 9 months (4-34) (Table 1). The majority of patients experienced one relapse (5.84%) while the rate for a second or third relapse was low (<1%) (Table 1). Univariate analysis identified a significantly increased risk of local recurrence in patients with tumor diameter ≥2 cm, poor differentiation, invasion beyond the subcutaneous layer, incomplete excision, ulceration and perineural involvement. Multivariate analysis also identified tumor diameter ≥ 2 cm, poor differentiation, incomplete excision and perineural invasion as significant independent risk factors for the development of local recurrence (Table 6).

Overall metastasis

Overall metastasis occurred in 84 patients (5.51%) after a median (25th-75th) period of 7 months (4-12). Fifteen patients presented with metastasis to surrounding tissue at their primary excision surgery and 69 developed postsurgical metastasis. Out of the last group, 57 patients (83.82%) developed lymph node metastasis, 7 (10.29%) distant metastasis and 4 (5.88%) combination of regional lymph node and distant metastasis. The main distant metastatic site was the lung, followed

Table 6. Univariate logistic regression model for positive SLNB, local recurrence, metastasis and death caused by cSCC in 1,524 patients, 2004-2014

Variables	*Odds Ratio (95% CI)*
Positive SLNB	
Older classification (max tumor diameter: ≥2/ < 2 cm)	11.64 (1.27, 106.72)
BWH classification (High/Low-risk)	2.8 (0.57, 13.83)
Our new classification (High/Low-risk)	2.29 (0.24, 22.09)
Local recurrence	
Older classification (max tumor diameter: ≥2/< 2 cm)	2.55 (1.69, 3.85)
BWH classification (High/Low-risk)	3.64 (2.39, 5.55)
Our new classification (High/Low-risk)	3.07 (1.96, 4.81)
Metastasis	
Older classification (max tumor diameter: ≥2/< 2 cm)	4.67 (2.77, 7.87)
BWH classification (High/Low-risk)	9.1 (5.75, 14.40)
Our new classification (High/Low-risk)	7.78 (3.99, 15.18)
Death caused by cSCC	
Older classification (max tumor diameter: ≥2/< 2 cm)	4.37 (2.38, 8.03)
BWH classification (High/Low-risk)	8.36 (4.91, 14.24)
Our new classification (High/Low-risk)	6.64 (3.13, 14.08)

by brain and then by the liver (Table 1). Of a total 58 metastatic patients 31(53.45%) were treated with radical lymph node dissection (cervical 77.42%, inguinal 12.90% and axillary 9.68%) +/- parotidectomy, with a median number of 8 infiltrated lymph nodes, and 27 conservatively treated (radiotherapy, chemotherapy or combination of both) (Table 3).

Univariate analysis revealed increased risk for metastasis in tumors characterized with diameter ≥2 cm, high-risk site, poor differentiation, invasion beyond subcutaneous tissue, incomplete excision, ulceration, perineural and lymphovascular involvement and recurrence. Multivariate analysis also identified diameter ≥2 cm, high-risk tumor site, poor differentiation, invasion beyond subcutaneous tissue, incomplete excision and perineural invasion along with local recurrence as independent risk factors for the development of metastasis (Table 7).

Survival analysis

During the follow-up period, 89 patients died (5.84% of the total study population); 60 patients (67.42%) had DCS after a median (25th-75th) period of months 1.91 (1.15-3.63) (Table 1). Of the patients with DCS, 41/60 (68.3%) had lymph node metastases, of which 9/60 had undergone SLNB. Three out of these 9 patients had negative SLNB while the rest were positive.

Univariate analysis of risk factors contributing to DCS: tumor diameter ≥2 cm, poor differentiation, invasion beyond subcutaneous tissue, incomplete excision, perineural invasion, lymphovascular in-

Table 7. Univariate and multivariate Cox regression models for recurrence

Recurrence	*Univariate analysis Crude HR (95% CI)*	*Multivariate analysis Adjusted HR (95% CI)*
Age (years)	1.01 (0.99, 1.03)	1.00 (0.99, 1.02)
Sex (Female / Male)	1.03 (0.69, 1.54)	1.05 (0.70, 1.58)
Primary tumor site (High / Low-risk)	1.19 (0.80, 1.76)	
Immunosuppresion (Yes / No)	0.71 (0.10, 5.08)	
Max tumor diameter (≥ 2 cm/< 2 cm)	2.65 (1.77, 3.95)	2.17 (1.44, 3.27)
Differentiation (Poor/moderate-well)	0.40 (0.27, 0.61)	0.55 (0.36, 0.84)
Invasion beyond subcutaneous fat (Yes/No)	2.89 (1.69, 4.92)	
Complete excision (No/Yes)	3.74 (2.30, 6.09)	3.03 (1.85, 4.97)
Ulceration (Yes/No)	1.94 (1.25, 3.01)	
Perineural invasion (Yes/No)	6.44 (3.59, 11.50)	4.24 (2.32, 7.75)
Lymphovascular invasion (Yes/No)	1.51 (0.48, 4.76)	
Associated inflammatory infiltrate (Yes/No)	1.19 (0.58, 2.45)	

HR: hazard ratio; CI: confidence interval

Table 8. Univariate and multivariate Cox regression models for metastasis

Metastasis	*Univariate analysis Crude HR (95% CI)*	*Multivariate analysis Adjusted HR (95% CI)*
Age (years)	1.01 (0.99, 1.03)	0.99 (0.97, 1.02)
Sex (Female / Male)	0.68 (0.39, 1.17)	0.74 (0.42, 1.31)
Primary tumor site (High/Low-risk)	1.95 (1.21, 3.12)	1.83 (1.11, 3.03)
Immunosuppresion (Yes/No)	2.41 (0.59, 9.84)	
Max tumor diameter (≥ 2 cm/< 2 cm)	3.71 (2.17, 6.35)	2.24 (1.27, 3.96)
Differentiation (poor/moderate-well)	0.27 (0.17, 0.43)	0.46 (0.27, 0.79)
Invasion beyond subcutaneous fat (Yes / No)	6.30 (3.74, 10.61)	2.10 (1.15, 3.82)
Complete excision (No / Yes)	4.77 (2.72, 8.34)	2.86 (1.59, 5.13)
Ulceration (Yes / No)	2.00 (1.15, 3.50)	
Perineural invasion (Yes / No)	8.55 (4.58, 15.97)	2.93 (1.45, 5.91)
Lymphovascular invasion (Yes / No)	6.86 (3.28, 14.35)	
Associated lymphatic infiltration (Yes / No)	0.91 (0.33, 2.49)	
Recurrence (Yes / No)	8.16 (4.99, 13.35)	3.65 (2.11, 6.30)

HR: hazard ratio; CI: confidence interval.

volvement, ulceration, and recurrence. Multivariate analysis verified tumor diameter ≥2 cm, poor differentiation, invasion beyond subcutaneous tissue, incomplete excision, recurrence and perineural invasion as independent risk factors for DCS (Table 8).

cSCC Staging systems

It is interesting that in BWH staging system, most patients (1.292 of 1.524;84.78%) presented with low-stage tumors (T1 or T2a) and a smaller group (232;15.22%) with high-stage (T2b or T3) tumors. Figure 2 presents relapse-free survival,

Table 9. Univariate and multivariate Cox regression models for death caused by cSCC

Death caused by cSCC	*Univariate analysis Crude HR (95% CI)*	*Multivariate analysis Adjusted HR (95% CI)*
Age (years)	1.01 (0.99, 1.04)	1.00 (0.98, 1.02)
Sex (Female / Male)	0.76 (0.43, 1.35)	0.73 (0.41, 1.32)
Primary tumor site (High / Low-risk)	1.55 (0.93, 2.58)	
Immunosuppresion (Yes / No)	2.96 (0.72, 12.13)	
Max tumor diameter (≥ 2 cm / < 2 cm)	4.21 (2.32, 7.66)	2.36 (1.26, 4.44)
Differentiation (poor/moderate-well)	0.24 (0.15, 0.41)	0.47 (0.27, 0.82)
Invasion beyond subcutaneous fat (Yes / No)	5.56 (3.14, 9.86)	2.11 (1.13, 3.92)
Complete excision (No / Yes)	5.16 (2.88, 9.26)	3.03 (1.64, 5.59)
Ulceration (Yes / No)	2.22 (1.20, 4.11)	
Perineural invasion (Yes / No)	9.32 (4.94, 17.58)	3.10 (1.54, 6.23)
Lymphovascular invasion (Yes / No)	7.40 (3.51, 15.61)	
Associated lymphatic infiltrate (Yes / No)	1.05 (0.38, 2.90)	
Recurrence (Yes / No)	8.98 (5.34, 15.11)	4.23 (2.39, 7.46)

HR: hazard ratio; CI: confidence interval

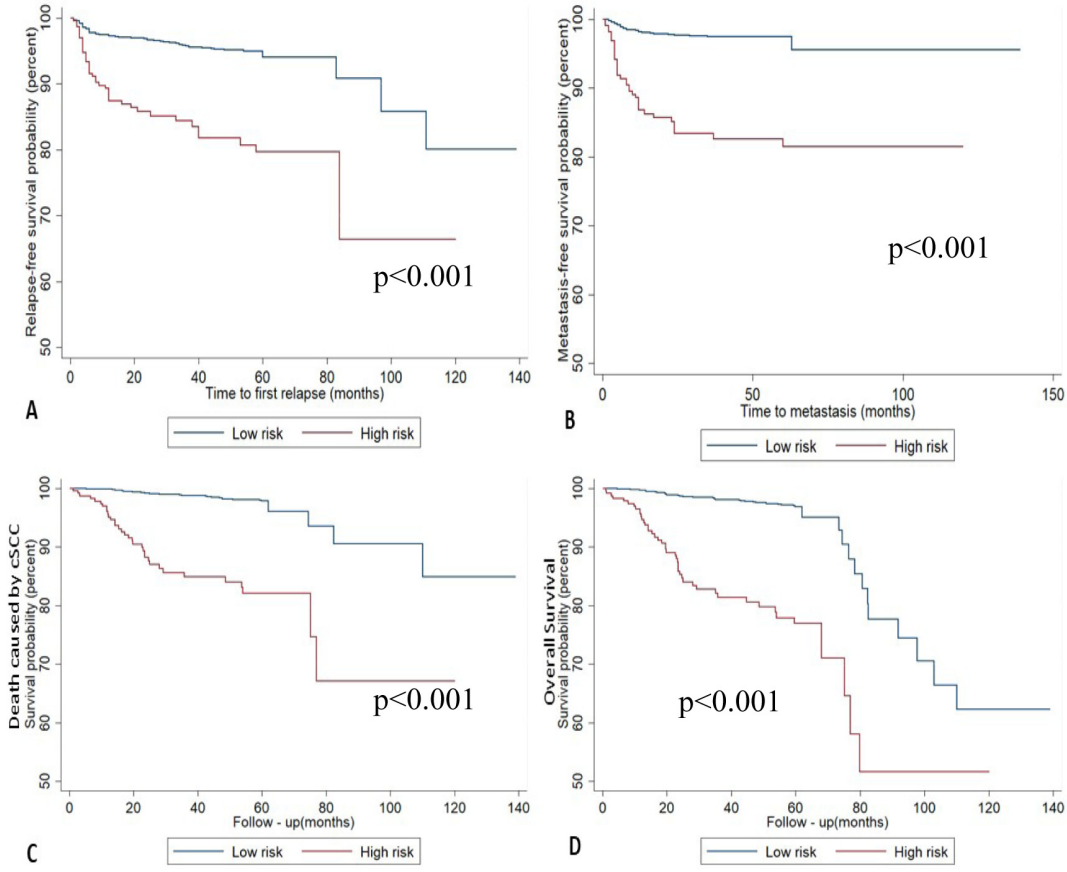

Figure 2. Kaplan-Meier curves for **(A)** local recurrence-free survival, **(B)** metastasis-free survival, **(C)** death caused by SCC-survival and **(D)** overall survival of different risk 1,524 patients with cSCC based on BWH staging system.

metastasis-free survival, death caused by SSCC-survival and overall survival of low-and high-risk patients based on BWM staging system. The same proportions reflected in our staging system with the majority of patients (1.240 of 1.524;81.36%) having low-risk tumors (≤ 2 risk factors) and the minority (284;18.64%) high-risk tumors (>2 risk factors) (Table 4).

Univariate analysis of each staging system separately with positive SLNB, LR, M, and DCS, revealed the last three parameters to be statistically significant. In concordance with the older system, diameter ≥ 2 cm was statistically significant for the prediction of positive SLNB (Table 9).

Discussion

Significance of SLNB in cSCCs

SLNB has been used since 1990 for melanoma staging as an effective tool for treatment and prognosis [9]. So far, SLNB in patients with high-risk cSCC has been reported only in case series, case reports [10] and different guidelines [11-13]. However, no homogeneous criteria exist, and all the various studies have led to a confusion of defining the high-risk factors for positive SLNB. As a model of care wherein a 10% risk threshold is generally sufficient for SLNB, the overall positivity rate for high-risk cSCC is about 14% [14]. Nowadays, SLNB is a significant tool with higher sensitivity (80%) in detecting lymph node metastasis compared to ultrasound (68%), magnetic resonance imaging (MRI) (75%) and computed tomographic (CT) (66%) [15,16]. False-negative SLNBs in high-risk cSCCs range from 10 to 33% [17].

In our study the rate of positive SLNB was high, about 25% (Table 3), with the highest globally registered 44% [18], whereas the false-negative rate was 11%. Despite their negative SLNB, two patients experienced postsurgical lymph node metastasis and 1 distant metastasis.

Regarding the different staging systems, the tumor diameter > 2 cm is the most important factor contributing to SLN metastasis as identified in our study as well [19]. However, Veness et al reported that 60% of lesions < 2 cm, but thicker than 4 mm, also had SLN metastasis [3]. Yet, due to the small number of patients who were enrolled, all studies were inadequate to prove the potential survival benefit of SLNB procedure [19]. Although we expected high-risk tumor site to play a statistically significant role in detecting micrometastases in regional lymph nodes, this was only shown in univariate analysis.

Analyzing the factors for local recurrence

Local recurrence was identified in 7.02% of our patients (Table 1), while in the literature ranged 6-28% [3,20-22]. Moreover, this study revealed the risk factors for recurrence, i.e. tumor diameter ≥ 2 cm, poor differentiation, incomplete excision and perineural involvement, in accordance with other studies [21,23]. The role of ulceration in staging of cSCCs in relation with recurrence was only proven significant in Cox univariate regression analysis. In cases of melanoma, along with Breslow thickness, ulceration plays a vital role in T2a/T2b categorization. Although ulceration has not been included in staging criteria, the depth or size of ulceration might be the key for identifying high-risk tumors in the future. Unfortunately, we were unable to prove the significance of tumor thickness statistically. Tumor thickness >6 mm is associated with increased risk for regional recurrence [21,24]. However, this finding is in accordance with the parameter of deep invasion, which was significant only in univariate Cox regression analysis, as an independent factor.

Analyzing the factors for metastasis

Current literature shows a range of metastatic rates for cSCC from 0.1 to 9.9%. In our study, 5.51% of our patients developed metastasis (Table 1) associated with the following: tumor diameter ≥ 2 cm, high-risk tumor site, poor differentiation, invasion beyond subcutaneous tissue, incomplete excision, perineural invasion and recurrence, also documented in many studies [13,25].

Furthermore, the association of ulceration with metastasis in univariate analysis is not yet described. Generally, immunosuppressed cSCC patients are sparse, as in our study, so larger prospective studies are required to prove their impact. In many studies tumor thickness is examined (especially > 5mm) [24,26], but it was inconsistently reported in our pathology reports. Hence its impact on metastasis was not analyzed. The multivariate analysis of Brantsch et al concluded that except for tumor diameter and high-risk site (ear), significant prognostic factors for metastases included increased tumor thickness and immunosuppression. Finally, poor differentiation as an independent risk factor, was verified in our study as well [7,27].

Analyzing the factors for overall survival (OS)

The majority of studies, including ours, has shown poor differentiation and perineural involvement are associated significantly with OS [21,27]. However, North et al and Jensen et al could not establish the differentiation grade as an independent prognostic factor for patient survival, possibly

due to their relatively small patient sample (40-60 patients) [28,29]. In addition, diameter ≥ 2 cm, invasion beyond subcutaneous tissue, incomplete excision and recurrence were independent risk factors for OS in our study.

Comparison of cSCC staging systems

Despite the difference in perspective of the BWH system, ours has achieved to identify a similar proportion of high-risk cSCCs and manage the patient treatment options similarly. Although both definitions seem to be quite relevant, in clinical practice there are some incongruities. This is caused by our inclusion of more clinical-pathological criteria (immunosuppression, incomplete excision, cSCC subtype, high-risk site). Thus, a low-risk characterized tumor in BWH system might be upstaged in our staging system due to the presence of more risk factors, such as immunosuppression. Overall, its worthy to highlight that the tumor diameter is the most significant factor in all classification systems (Table 9).

Limitations / comments

Greek population itself, due to its small size, may present some limitations for the identification of cSCCs prognostic factors. Until now, official population-based incidence of cSCCs in Greece is not available. However, this study achieved to collect a sample of 1,524 patients, comparable to ideally organized cohorts from USA [6,8]. Moreover, this investigation represents the first large sized study of cSCC able to yield risk factors and allowing multivariate analysis to be performed. Furthermore, it is one of the few studies that three different staging systems are compared and suggests simultaneously a modified one.

Although this is the most extensive study to date of cSCC outcomes in Southern Europe, it may have underestimated some potentially critical prognostic factors such as immunosuppression and tumor depth. Future studies may estimate whether millimeter depth or tissue level of invasion has greater prognostic significance.

Nine out of 36 patients (25%) had positive SLNB. This is much higher than 5-10%, usually reported in melanoma studies [30], indicating that SLNB may be underutilized in high-risk cSCCs. However, our sample is small, and the selection of patients may not have included all high-risk cSCC cases. Larger studies evaluating the impact of SLNB on cSCC outcomes are needed.

Finally, in this retrospective study, follow-up procedure was not uniform. The majority was examined by dermatologists or plastic surgeons, whereas other patients were interviewed on phone by physician-researcher. Because of this, local recurrence may have been underreported. On the contrary, nodal metastasis, distant metastasis or death caused by cSCC are unlikely to have been underestimated as they are mainly diagnosed by medical experts.

Conclusions

Although the different staging systems suggest distinct criteria with specific high-risk factors, there are no supporting data for a unique valid definition. Thus, our study attempted to examine the independent effect of each factor and interaction effect between them on the risk of recurrence, metastasis, positive SLNB and death caused by cSCC. Unfortunately, no study until now has had the sufficient power to establish uniform guidelines and the management of these patients is not homogeneous. So, development of reliable prognostic models will aid clinicians design clinical trials, target nodal staging, make reasonable treatment options as well as develop adjuvant therapies in the course of disease. Finally, although high-risk cSCC is rarely fatal, it has significant adverse effects on public health. The high medical costs and the compromised quality of life by devastating aesthetic and psychological sequelae or functional impairment are some of the severe consequences in this regard.

Acknowledgement

Dr Emmanouil Dimonitsas would like to thank Onassis Foundation for the scholarship. The scientific paper was supported by the Onassis Foundation-Scholarship ID: GZM 019-1/2016-2017. The financial assistance was in the context of PhD Thesis.

Conflict of interests

The authors declare no conflict of interests.

References

1. Karia PS, Han J, Schmults CD. Cutaneous squamous cell carcinoma: estimated incidence of disease, nodal metastasis, and deaths from disease in the United States, 2012. J Am Acad Dermatol 2013;68:957-66.
2. Stratigos AJ, Garbe C, Dessinioti C et al. European interdisciplinary guideline on invasive squamous cell carcinoma of the skin: Part 1. epidemiology, diagnostics and prevention. Eur J Cancer 2020;128:60-82.
3. Veness MJ, Palme CE, Morgan GJ. High-risk cutaneous squamous cell carcinoma of the head and neck: results from 266 treated patients with metastatic lymph node disease. Cancer 2006;106:2389-96.
4. Takahashi A, Imafuku S, Nakayama J, Nakaura J, Ito K, Shibayama Y. Sentinel node biopsy for high-risk cutaneous squamous cell carcinoma. Eur J Oncol 2014;40:1256-62.
5. Parikh SA, Patel VA, Ratner D. Advances in the management of cutaneous squamous cell carcinoma. F1000prime reports 2014;6.
6. Schmults CD, Karia PS, Carter JB, Han J, Qureshi AA. Factors predictive of recurrence and death from cutaneous squamous cell carcinoma: a 10-year, single-institution cohort study. JAMA Dermatol 2013;149:541-7.
7. Rowe DE, Carroll RJ, Day CL Jr. Prognostic factors for local recurrence, metastasis, and survival rates in squamous cell carcinoma of the skin, ear, and lip: implications for treatment modality selection. J Am Acad Dermatol 1992;26:976-90.
8. Karia PS, Jambusaria-Pahlajani A, Harrington DP, Murphy GF, Qureshi AA, Schmults CD. Evaluation of American Joint Committee on Cancer, International Union Against Cancer, and Brigham and Women's Hospital tumor staging for cutaneous squamous cell carcinoma. J Clini Oncol 2014;32:327.
9. Morton DL, Thompson JF, Cochran AJ et al. Final trial report of sentinel-node biopsy versus nodal observation in melanoma. N Engl J Med 2014;370:599-609.
10. Rakheja M, Radhakrishnan R, Solomon MC. Sentinel lymph node biopsy in oral squamous cell carcinoma: Ensuing from elective to selective. J Res Med Den Sci 2014;2:5-10.
11. Bonerandi J, Beauvillain C, Caquant L et al. Guidelines for the diagnosis and treatment of cutaneous squamous cell carcinoma and precursor lesions. J Eur Acad Dermatol Venereol 2011;25:1-51.
12. Breuninger H, Eigentler T, Bootz F et al. Brief S2k guidelines–cutaneous squamous cell carcinoma. JDDG: J Deutsch Dermatol Gesellschafts 2013;11:37-45.
13. Miller SJ. The National Comprehensive Cancer Network (NCCN) guidelines of care for nonmelanoma skin cancers. Dermatol Surgery 2000;26:289-92.
14. Kwon S, Dong ZM, Wu PC. Sentinel lymph node biopsy for high-risk cutaneous squamous cell carcinoma: clinical experience and review of literature. World J Surg Oncol 2011;9:80.
15. Hodder S, Evans R, Patton D, Silvester K. Ultrasound and fine needle aspiration cytology in the staging of neck lymph nodes in oral squamous cell carcinoma. Br J Oral Maxillof Surg 2000;38:430-6.
16. Kabir S, Schmults CD, Ruiz ES. A review of cutaneous squamous cell carcinoma epidemiology, diagnosis, and management. Int J Cancer Manag 2018;11:e60846.
17. Krediet J, Beyer M, Lenz K et al. Sentinel lymph node biopsy and risk factors for predicting metastasis in cutaneous squamous cell carcinoma. Br J Dermatol 2015;172:1029-36.
18. Renzi C, Caggiati A, Mannooranparampil T et al. Sentinel lymph node biopsy for high risk cutaneous squamous cell carcinoma: case series and review of the literature. Eur J Surg Oncol 2007;33:364-9.
19. Schmitt AR, Brewer JD, Bordeaux JS, Baum CL. Staging for cutaneous squamous cell carcinoma as a predictor of sentinel lymph node biopsy results: meta-analysis of American Joint Committee on Cancer criteria and a proposed alternative system. JAMA Dermatol 2014;150:19-24.
20. Clayman GL, Lee JJ, Holsinger FC et al. Mortality risk from squamous cell skin cancer. J Clin Oncol 2005;23:759-65.
21. Kyrgidis A, Tzellos TG, Kechagias N et al. Cutaneous squamous cell carcinoma (SCC) of the head and neck: risk factors of overall and recurrence-free survival. Eur J Cancer 2010;46:1563-72.
22. Oddone N, Morgan GJ, Palme CE et al. Metastatic cutaneous squamous cell carcinoma of the head and neck: the Immunosuppression, Treatment, Extranodal spread, and Margin status (ITEM) prognostic score to predict outcome and the need to improve survival. Cancer 2009;115:1883-91.
23. Liégeois NJ, Seo SJ, Olbricht S. Squamous cell carcinoma. Evidence-Based Dermatology Oxford: Blackwells 2008:248-55.
24. Brantsch KD, Meisner C, Schönfisch B et al. Analysis of risk factors determining prognosis of cutaneous squamous-cell carcinoma: a prospective study. Lancet Oncol 2008;9:713-20.
25. Brougham ND, Dennett ER, Cameron R, Tan ST. The incidence of metastasis from cutaneous squamous cell carcinoma and the impact of its risk factors. J Surg Oncol 2012;106:811-5.
26. Haisma MS, Plaat BE, Bijl HP et al. Multivariate analysis of potential risk factors for lymph node metastasis in patients with cutaneous squamous cell carcinoma of the head and neck. J Am Acad Dermatol 2016;75:722-30.
27. Brinkman JN, Hajder E, van der Holt B, Den Bakker MA, Hovius SE, Mureau MA. The effect of differentiation grade of cutaneous squamous cell carcinoma on excision margins, local recurrence, metastasis, and patient survival: a retrospective follow-up study. Ann Plastic Surg 2015;75:323-6.
28. North JH Jr, Spellman JE, Driscoll D, Velez A, Kraybill WG, Petrelli NJ. Advanced cutaneous squamous cell carcinoma of the trunk and extremity: analysis of prognostic factors. J Surg Oncol 1997;64:212-7.
29. Jensen V, Prasad AR, Smith A et al. Prognostic criteria for squamous cell cancer of the skin. J Surg Res 2010;159:509-16.
30. Berk DR, Johnson DL, Uzieblo A, Kiernan M, Swetter SM. Sentinel lymph node biopsy for cutaneous melanoma: the Stanford experience, 1997-2004. Arch Dermatol 2005;141:1016-22.