Histological predictors of outcome for cutaneous squamous cell carcinoma in renal transplant patients: A case-control study

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Background: Cutaneous squamous cell carcinoma is a significant cause of morbidity for immunosuppressed patients such as organ transplant recipients; however, histological parameters which predict the likelihood of tumor progression are typically based on general population studies in which immunosuppressed patients represent only a small fraction of cases.

Objectives: To determine the histological parameters which have independent prognostic value for cutaneous squamous cell carcinoma arising in renal transplant recipients.

Methods: Case-control study incorporating a retrospective blinded histological review of 70 archived specimens of cutaneous squamous cell carcinoma diagnosed in renal transplant recipients, comprising 10 cases where the tumor had progressed and 60 controls.

Results: Progression was significantly associated with head and neck location, size, depth, poor histological grade, perineural invasion (including small caliber perineural invasion), lymphovascular invasion, and a desmoplastic growth pattern.

Limitations: The retrospective nature and the low number of cases compared to controls.

Conclusion: In immunosuppressed patients both small caliber perineural invasion and a desmoplastic growth pattern may also have prognostic significance in addition to other histological parameters already recognized in formal staging schemes. (JAAD Int 2024;15:51-8.)

Key words: carcinoma; logistic models; retrospective studies; skin neoplasms; squamous cell; transplant recipients.

INTRODUCTION

Nonmelanoma skin cancer, predominantly cutaneous squamous cell carcinoma (cSCC), is the most common malignancy to occur in recipients of organ transplants.[1] This represents a significant source of morbidity for these patients, with an estimated

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cumulative incidence for cSCC of 45% at 11 years posttransplantation, rising to 70% at 20 years posttransplantation.[2] Compared to the general population, cSCC in transplant recipients occurs at a rate 65 times higher,[3] with an increased risk of both local recurrence and metastasis as well as a relative risk of tumor-related death of 52.[4-8] Patients in Australia are at a particularly high risk, with a relative risk of 3.6 compared to European posttransplant populations.[2]

The management of cSCC in transplant patients is the same as in nontransplant patients, with the mainstay of therapy being complete excision.[1] A number of adjuvant treatments may also be considered in patients deemed at higher risk[9,10] and sentinel lymph node biopsy, while not yet established as a routine procedure, may also have some benefit in selected cases.[11-14] The use of these therapies requires a balance between the risk of the intervention and the risk of progression of the tumor. Histological parameters which correlate with tumor progression are of great value in this decision-making process. While some histological features are incorporated into formal staging systems,[15] these are typically based on general population studies in which immunosuppressed patients represent only a small fraction of cases.[16,17] It is possible that some parameters which do not reach statistical significance in general population studies may have more significance in patients with compromised immunity. To identify any such parameters we used a retrospective case-control approach to review archived histopathological specimens of cSCC from renal transplant recipients.

CAPSULE SUMMARY

- Some histological parameters associated with cutaneous squamous cell carcinoma progression are not routinely reported but may have significance in patients with compromised immunity.
- Small caliber perineural invasion and desmoplasia are predictive of outcome in patients with immunosuppression. Practitioners should be aware that these findings are of potential significance in this context.

METHODS

The electronic records of the Department of Anatomical Pathology, PathWest (Perth, Western Australia) were searched for cases of cSCC occurring in patients who had received a renal transplant between 2010 and 2017. We did not require the patients to be on active immunosuppression at the time of tumor detection. This study utilized a case-control design, with the study population defined as all renal transplant recipients who had been diagnosed with an invasive cSCC (at least 1-mm thickness) in which the entire lesion could be assessed. The clinical records for each patient were examined for any evidence of tumor progression, defined as locoregional recurrence, spread to regional lymph nodes or distant metastases. The "cases" were defined as lesions where there was evidence of progression within 24 months of diagnosis (prior studies of cSCC in immunosuppressed patients have reported a median time to progression of 6 months).[18] Lesions for which metastases were present at the time of initial diagnosis were regarded as cases. The "controls" were defined as lesions where there was no evidence of further progression with a follow-up time of at least 24 months. All but one of the tumors designated as "cases" were completely excised with clear histological margins after the initial surgical intervention. The remaining case was cleared with a subsequent procedure.

Data regarding the site and diameter of the primary tumor were retrieved from the filed histological report. Hematoxylin and eosin stained sections were retrieved from the excision specimen (either elliptical excisional biopsy or punch excision) and the following histological parameters were assessed independently by a single observer (N.T.H.) who was blinded to the clinical outcome.

1. Histological grade (2-tiered, comprising well/moderately differentiated tumors and poorly differentiated tumors. Any lesions demonstrating a sarcomatoid morphology were coded as poorly differentiated).
2. Maximum superficial to deep thickness of the tumor.
3. Depth (from the top of the granular layer in adjacent nonelevated epidermis, to the deepest tumor cell).
4. Ulceration (including width in mm).
5. Tumor budding.[19,20]
6. Perineural invasion, including the maximum size and anatomical location of the involved nerve.
7. Lymphovascular invasion.
8. Desmoplasia.[21]
9. Associated actinic keratosis/squamous carcinoma in situ.
10. Associated Bowen disease (defined as a distinct pattern of squamous carcinoma in situ characterized by keratinocyte dysplasia involving the full thickness of the epidermis with areas of

Abbreviations used:

AJCC: American Joint Committee on Cancer
cSCC: cutaneous squamous cell carcinoma

pagetoid growth and at least focal sparing of the basal layer).

11. Mitotic count (mitotic figures/mm^2).

Statistical analysis was performed using SPSS software (version 25 for Windows, SPSS Inc). Differences in participant characteristics between the cases (n = 10) and controls (n = 60) were determined using an independent samples t test (parametric) for normally distributed continuous variables, Mann-Whitney U test (nonparametric) for nonnormally distributed continuous variables, and χ^2 test for independence when both variables were categorical. After initially checking for high intercorrelations among predictor variables, a logistic regression model was used to determine which clinical and/or histological observations (categorical and continuous variables) best predicted the cases from the controls.

The project was approved by the Government of Western Australia Department of Health Human Research Ethics Committee (RGS931).

RESULTS

The initial review of histological reports identified 74 cases meeting our inclusion criteria, from 59 patients. From these 4 cases were excluded: 2 because the slides were unavailable for review, 1 because the invasive component was judged to be less than 1-mm depth and 1 because on review the lesion was regarded as most likely representing squamous carcinoma in situ only. This left a total study set of 70 cases, comprising 10 cases and 60 controls. The median time to tumor progression after initial diagnosis for the cases was 7 months (range 0-20). The median follow-up time for the controls was 52 months (range 25-124).

Table I summarizes the clinical and histological features of the cases compared with the controls. There was no significant difference between the 2 groups regarding age at diagnosis or gender, with the majority of patients being male in keeping with previous studies investigating clinical parameters associated with cSCC development.[8,22] All tumors which progressed occurred in the head and neck area, which was significantly different to the controls in which 45% occurred outside this anatomical region (P = .018). The tumors which progressed were significantly larger than those within the control group, regardless of whether macroscopic dimension (P = .007), thickness (P = .001), or depth (P < .001) were considered. However, there were no significant differences between the 2 groups regarding the presence or size of tumor ulceration. More lesions were regarded as poorly differentiated in the cases group compared to the controls (40% vs 10%, P = .043). The presence of perineural invasion was strongly associated with an adverse outcome (P = .001), with tumor progression resulting in all but one of these cases. The details of these cases are outlined in Table II and an example is illustrated in Fig 1. It is worth noting that for several of these tumors the involved nerve was low-caliber (<0.1-mm diameter), and 2 of the tumors which subsequently progressed would have been regarded as stage pT1 by current American Joint Committee on

Table I. Summary of the clinical and histological findings of cases and controls in this study

Characteristic	Case (n = 10)	Control (n = 60)	P value
Age (y)	64.8 ± 13.1	59.8 ± 8.0	.271*
Gender (male)	9 (90%)	50 (83%)	1.00†
Site			
Head and neck	10 (100%)	33 (55%)	**.018**†
Nonhead and neck	0	27 (45%)	
Tumor size			
Max dimension (mm)‡	35.0 (11.5-45)	10.0 (7.0-15.0)	**.007**§
Thickness (mm)‡	10.0 (4.0-15.25)	2.5 (2.0-4.19)	**.001**§
Depth (mm)‡	6.5 (3.88-10.5)	1.8 (1.5-2.5)	**<.001**§
Ulceration			
Ulceration (Y/N)	9 (90%)	36 (60%)	.14†
Ulcer dimension (mm)‡	13.0 (1.5-30.0)	5.0 (3.0-8.0)	.20§
Grade			
Well/mod diff	6 (60%)	54 (90%)	**.043**†
Poorly diff	4 (40%)	6 (10%)	
Other histological features			
Budding (Y/N)	4 (40%)	12 (20%)	.32†
PNI (Y/N)	6 (60%)	1 (1.7%)	**<.001**†
LVI (Y/N)	3 (30%)	2 (3.3%)	**.018**†
Desmoplasia (Y/N)	9 (90%)	19 (31.7%)	**.002**†
AK/SCCIS (Y/N)	5 (50%)	36 (60%)	.80†
Bowens (Y/N)	1 (10%)	16 (27%)	.46†
Mitotic count (mf/mm^2)‡	7.5 (4.75-14.5)	5.0 (2.25-9.0)	.11§

Bold values indicates P value <.05.
AK/SCCIS, Actinic keratosis/Squamous carcinoma in situ; *LVI,* lymphovascular invasion; *PNI,* Perineural invasion.
*Independent samples t test.
†χ^2 for independence.
‡Median (interquartile range).
§Mann-Whitney U test.

Table II. Clinical details, tumor characteristics, size/location of involved nerves and clinical outcome of the 6 study cases which showed histological perineural invasion

Gender and age (y)	Site	Tumor maximum dimension (macroscopic, mm)	Depth of invasion (mm)	pT stage (AJCC eighth ed.)	Histological grade	Diameter of largest involved nerve (mm)	Location of involved nerve	Outcome
Male, 68	Lateral canthus	4	1.5	pT1	Poorly differentiated	0.09	Dermis	Local recurrence after 15 mo.
Male, 82	Scalp	85	7	pT3	Poorly differentiated (spindle cell/sarcomatoid)	0.05	Dermis	Cutaneous and lymph node metastases after 1 mo.
Male, 79	Ear	45	17	pT3	Well/moderately differentiated	0.07	Dermis	Lymph node metastases identified at time of primary excision.
Male, 81	Scalp	40	12	pT3	Well/moderately differentiated	0.12	Subcutis	Local recurrence after 7 mo.
Male, 66	Forearm	20	2.5	pT2	Well/moderately differentiated	0.02	Dermis	No progression after 25 mo.
Male, 52	Cheek	8	3.5	pT1	Well/moderately differentiated	0.07	Dermis	Local recurrence after 9 mo.
Male, 54	Scalp	45	10	pT3	Well/moderately differentiated	0.10	Dermis	Local recurrence and lymph node metastases after 7 mo.

AJCC, American Joint Committee on Cancer.

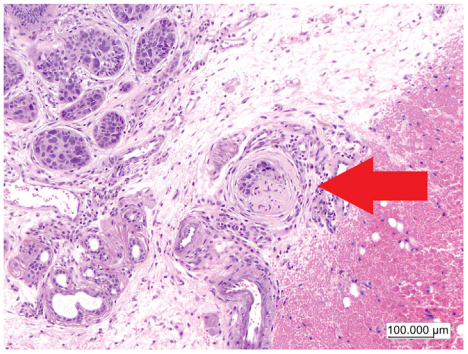

Fig 1. Cutaneous squamous cell carcinoma invading the perineural space (*red arrow*). This was a poorly differentiated cutaneous squamous cell carcinoma excised from the lateral canthus of a 68-year-old male. The involved nerve measured 0.09 mm in diameter and was located in the dermis. The tumor recurred locally after 15 months. (Hematoxylin and eosin; original magnification 100×).

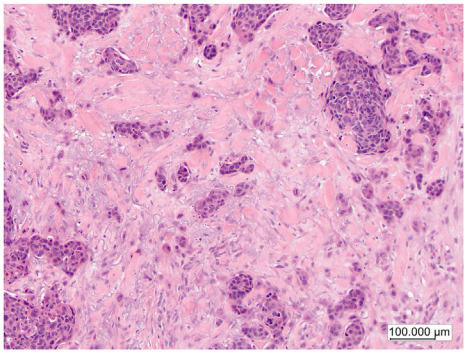

Fig 2. Cutaneous squamous cell carcinoma showing a desmoplastic growth pattern. The epithelial nests, cords or single epithelial cells lie within a sclerotic or fibroblastic stroma. This pattern needed to represent at least 30% of the tumor volume to be recorded as present. (Hematoxylin and eosin; original magnification 100×).

Cancer (AJCC) staging criteria (eighth edition).[15] Lymphovascular invasion (P = .018) and a desmoplastic growth pattern (P = .002, illustrated in Fig 2) were also significantly associated with tumor progression. No significant differences between the 2 groups were identified for tumor budding, the presence of intraepidermal dysplasia (actinic keratosis, squamous carcinoma in situ, or Bowen disease) or mitotic counts.

Table III summarizes the logistic regression model. Given the small sample size of 10 cases, the number of predictors included in the logistic regression was limited to those variables where there was a highly significant difference (P < .02) between the cases and controls. The site was not included as all cases in this analysis were head and neck only, and thickness was included, but not maximum dimension or depth as thickness was significantly correlated to both (maximum dimension, r = 0.61, P < .001; depth, r = 0.83, P < .001). The Hosmer and Lemeshow Goodness of Fit Test significance for this combination of variables was 0.995, which indicated support for the model. The χ^2 value was 44.07 with 4 degrees of freedom. Depth (odds ratio = 4.74; 95% CI, 1.47-15.28; P = .009), perineural invasion (odds ratio = 879.89; 95% CI, 2.60-297,286.25; P = .022) and desmoplasia (odds ratio = 112.55; 95% CI, 1.14-11,072.20; P = .044) were the variables that contributed significantly to the predictive ability of the model.

Table III. Logistical regression model incorporating depth of invasion, perineural invasion, desmoplasia, and lymphovascular invasion

	P value	Odds ratio	95% CI for odds ratio
Depth of invasion	.009	4.74	1.47-15.28
Perineural invasion	.022	879.89	2.60-297286.25
Desmoplasia	.044	112.55	1.14-11072.20
Lymphovascular invasion	.380	6.11	0.11-345.81

Hosmer and Lemeshow Test Goodness of Fit Test significance 0.995.
χ^2 44.07 (4 degrees of freedom).

DISCUSSION

Accurate staging of cSCC in immunosuppressed patients is essential for determining whether further therapeutic interventions are required after the initial excision. Early staging systems for cSCC were criticized due to a lack of discriminatory ability with regard to determining risk of progression, but more recent iterations have shown improved correlation with outcome.[23-26] While earlier schemes focused predominantly on size and features of advanced spread (such as bony invasion), more recent schemes have included histological parameters such as depth of invasion, poor differentiation, and perineural invasion.[15] While these schemes show broad correlation with outcome in organ transplant recipients,[8,18] a background of immunosuppression is in itself a "high-risk" feature,[16] and some have suggested that it should be included as such in formal staging schemes.[27-29] The objective of this study was to investigate the correlation of histological parameters with tumor progression in an immunosuppressed population, as it seemed plausible that some criteria which were not independently predictive at a population level may have more significance in this high-risk group.

Our retrospective case-control design identified a number of factors that were significantly associated with tumor progression when analyzed in a univariate fashion. Firstly, all of the tumors which progressed were located in the head and neck region. This was not particularly surprising, as areas such as the central face, lips, periorbital region, and ear have all been recognized as high-risk sites in previous studies,[7,16,17,28] including studies looking specifically at transplant recipients.[8] A similarly unsurprising finding was that larger tumors were significantly associated with a risk of progression, whether this was measured as maximum macroscopic dimension, thickness, or depth. Tumor size and depth of invasion are well established as independent prognostic factors for cSCC, with evidence supporting thresholds of 2 cm in diameter and 6 mm in depth (or deeper than the subcutaneous fat) as being related to an increased chance of metastasis.[7,8,16,17,25,30-35] Both of these are now formalized as determinants for T-staging within the current AJCC staging system (eighth edition), with depth defined as the measurement in millimeters from the granular layer of adjacent normal skin to the base of the tumor.[15] In reality we suspect that in practice there may be some variation in whether pathologists record the depth or thickness.[36,37] While it was reassuring that both depth and thickness were relatable to outcome, it is worth noting the pitfalls of using thickness, which include overestimating the risk tumors characterized by a significant exophytic component as well as invalidating the 6-mm depth cut-off for upstaging to pT3.

Perineural invasion showed a particularly strong correlation with tumor progression, and in our logistical regression model represented one of the stronger contributors to its predictive ability. Perineural invasion is well recognized to be associated with adverse outcome in cSCC;[7,17,31-33,35,38,39] however, several recent studies have suggested that this association is only significant when larger nerves (0.1-mm diameter or greater) are involved.[38,40] Thus, the AJCC eighth edition staging system only incorporates perineural invasion as a risk factor for larger nerves (as defined above) or those located deeper than the dermis (which are typically larger in caliber anyway). However, in our cohort many of the tumors which progressed were associated with small caliber perineural involvement only, and indeed 2 of these cases would have been staged as pT1 under the current AJCC system (Table II). Thus, our results suggest that in the setting of immunosuppression even low caliber perineural invasion is a significant prognostic factor and should not be ignored. It is worth noting that in the study by Carter et al, there were still adverse outcomes in patients with small caliber perineural invasion, in most cases associated with other recognized risk-factors; however, they did not find a significant association with immunosuppression.[38]

The other histological parameters that demonstrated a statistically significant association with tumor progression were poor differentiation, lymphovascular invasion, and desmoplasia. All of these have been previously shown to be associated with worse outcomes,[7,8,16,17,31,33,35,41] and although they are recognized as "high-risk" features in the AJCC staging system, none are formally incorporated as determinants of the T-category.[15] Desmoplasia was also a significant contributor to the logistic regression model (poor differentiation was not included due to the low number of cases showing this feature in each group). Histological grading and desmoplasia are somewhat subjective features, making them less useful when a homogeneous approach to staging is desired. In particular, the grading of cSCC is still technically based on the scheme outlined by Broders in 1932,[42] and while it has been included in previous AJCC editions as well as the alternative Brigham and Women's Hospital staging schemes, its inclusion attracted criticism due to the lack of explicit definitions for what constituted a poorly differentiated tumor, given that a range of morphological subtypes (sarcomatoid, basaloid, adenosquamous, carcinosarcoma) could potentially be included.[29] Tumors showing a desmoplastic growth pattern as defined by Breuninger et al[21] have previously been associated with an increased risk for recurrence, including in multivariate analysis.[16] There may be some overlap morphologically with "poorly differentiated" tumors, and some authors have suggested that desmoplasia may be a more useful criterion for formal staging schemes than grading.[16] This histological pattern is likely representative of a more aggressive tumor phenotype, and further investigations focusing on this possibility may yield important biological insights. We also investigated the presence of so-called "tumor budding" as potentially another indicator of an "aggressive" tumor phenotype. While there is evidence that this feature may be a useful prognostic indicator for squamous cell carcinoma in the oral cavity, nasopharynx, and larynx,[43] there are only limited data supporting its role in cSCC,[19,20] and we found no significant difference in this finding between our cases and controls.

The main weaknesses of our study were the retrospective nature and the low number of cases compared to controls. The latter factor is reflected in the very wide confidence intervals associated with the variables included in the logistic regression model. We felt that this study would be

underpowered with regard to assessing other potentially useful variables, such as measured histological clearance of margins or time to recurrence/metastasis. Validation of this model in a larger set of tumors will be a logical progression from this study, and would allow evaluation of these factors as well. However, a major strength is the formal histological review of each case, undertaken in a blinded fashion. The use of a single observer meant we were unable to assess interobserver reliability of the histological parameters assessed, and this would be important to determine going forward. Nonetheless, at least of the more important (tumor depth, histological grade, perineural invasion) have been shown to have good interobserver concordance in previous studies.[34]

In summary, we identified a number of parameters that are associated with cSCC tumor progression in high-risk immunosuppressed renal transplant recipients. While all of these have been associated with adverse outcome in prior studies, not all are formally included in current staging schemes. Small caliber perineural invasion and a desmoplastic growth pattern in particular could potentially be overlooked. Pathologists should be aware of the increased risk in immunosuppressed patients, warranting a more comprehensive assessment of histological specimens with particular attention paid to these parameters.

Conflicts of interest

None disclosed.

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