Histopathologic Features Predictive of Aggressiveness of Post-transplant Cutaneous Squamous-cell Carcinomas

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Abstract. *Background. Squamous cell carcinomas (SCC), the commonest malignancies developing in organ-transplant recipients (OTR), may behave aggressively. We searched for pathological features of post-transplant SCC that could predict an aggressive outcome early. Materials and Methods. We pathologically examined 34 SCC developed in OTR that developed later recurrences/metastases, and compared them with 25 non-aggressive SCC excised from the same OTRs over the same period of time for features believed to predict an aggressive outcome (tumour size and thickness, ulceration, deep tissue invasion, mitotic rate, differentiation, peritumoural infiltrate density, acantholysis, perineural and lymphovascular invasion). Results. A statistically significant difference was found for the level of tumour invasion (Clark), as 58% (18/34) of aggressive SCCs (vs 24% (6/25) of non-aggressive SCCs) were of levels IV or V. Conclusion: Post-transplant SCCs with a Clark level of IV or V are associated with a higher risk for recurrence and metastasis and call for a close patient follow-up.*

Non-melanocytic skin cancer, including mainly basal cell carcinomas and squamous cell carcinomas (SCC), are the most common malignancies developing in organ-transplant recipients (OTRs). The risk of developing SCC is increased 60 to 100-fold in OTRs compared with the non-immunosuppressed population, so that in the USA and Western Europe, 40-60% of OTRs develop cutaneous SCC at 20 years post-transplantation, with rates being even higher in Australia (1-3). Because of their multiplicity and their potentially aggressive behaviour, cutaneous SCCs entail significant morbidity and mortality in OTRs, and have become a major health concern in this patient population. Although most cutaneous SCCs can be cured with surgical excision with adequate margins, a minority of SCC, have an aggressive course, giving rise to local recurrences and metastases (regional or distant). This occurs in 3-8% and 1.9-10% of cases, respectively (4-6), and results in a mortality rate of 2-5% for all cutaneous SCCs (6, 7). Some clinicopathological features are considered to be positively associated with aggressive behaviour of SCC in terms of local recurrence and metastasis. They include: tumour location (namely the ear), macroscopic (horizontal) tumour size (>2 cm), regional node disease, recurrent SCC, microscopic tumour thickness (>2 mm), Clark level (≥IV), invasion of deep (subcutaneous) tissues (muscles, bone or cartilage), poor differentiation and intravascular and perineural invasion (4-12). Some authors have also claimed intratumoural acantholysis to be an adverse pathological finding (13), although this contention has not been confirmed by others (14). Immunosuppression is believed to be a risk factor for metastasis in SCC (3, 4), as SCCs developing in OTRs reportedly exhibit more aggressive pathologic features than their counterparts in immunocompetent patients (15). Most OTRs develop multiple SCCs (1, 16), some of which may be aggressive (17). The reasons why some primary SCCs become aggressive, despite developing simultaneously with non-aggressive tumours in the same OTR, are not well understood, although this clinical observation strongly suggests that some SCCs carry an intrinsically high risk for an unfavourable outcome. Early recognition of such high-risk SCC is important in order to undertake a tailored management, including adequate tumour treatment and subsequent close patient surveillance.

Some previous works have studied the pathological features of SCCs developing in OTRs (13, 18-20), however, as far as we know, no study has yet specifically compared aggressive with non-aggressive SCC in OTRs in order to search for pathological features that could early predict an aggressive outcome of SCC developing in this patient group. This reason prompted us to perform the present study.

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Materials and Methods

Study design. A pathological study was performed on the initial surgical specimens of SCCs that had been excised from OTRs followed up in our specialized outpatient Dermatology Clinic and that proved upon follow-up to have an aggressive behaviour, defined as the development of local recurrences or metastases (regional or distant), despite an initial surgical excision with tumour-free margins. For SCCs that had undergone multiple excisions (because of recurrences), only the initial SCC was studied.

Pathological examination. Representative haematoxylin and eosin-stained tissue sections of each SCC were re-examined for the following features: macroscopic (horizontal) tumour size; microscopic tumour thickness (measured from the granular layer of the overlying epidermis to the deepest tumour mass); Clark level of invasion (II: papillary dermis, III: upper reticular dermis, IV: whole dermis, V: hypodermis and beyond); invasion of subcutaneous tissues (such as cartilage, bone and muscle); degree of tumour differentiation (scored as good, moderate or poor according to the percentage of well-differentiated tumour cells); presence of ulceration, acantholysis, perineural and lympho-vascular invasion; number of mitoses (total and atypical) counted in five high-power fields; and density of peritumoural cell infiltrate (scored as absent/sparse, moderate or dense). In parallel with these aggressive SCCs (A-SCCs), as controls we studied a group of non-aggressive SCCs (C-SCCs): these were tumours excised from the same group of OTRs during the six months preceding or following excision of the A-SCCs that during the same follow-up period as the A-SCCs did not develop recurrences or metastases. The pathological re-examination of the tissue sections was performed by two of us (JK, GK) in a blinded fashion as to the patient and the nature of the SCC (aggressive or not).

Statistical analysis. This was performed with the Wilcoxon test for quantitative values, and the Fishers' (or the χ^2) test for qualitative values. A *p*-value of 0.05 or less was considered significant.

Results

In the cohort of approximately 4300 OTRs followed-up in our Department since 1991, 375 patients developed a total of 1244 cutaneous SCCs. A-SCCs (as defined above) were identified through the medical files in 43 OTRs: 19 of them developed local recurrences of their SCC, 16 developed recurrences followed by metastases, and eight patients developed metastatic SCC without preceding tumour recurrence. A total of 34 initial SCCs were available to us for pathological re-examination (nine aggressive SCCs had been excised at other hospitals and the corresponding slides could not be retrieved). These had developed in 32 Caucasian OTRs who were mostly renal-transplant patients receiving a triple immunosuppressive treatment consisting of steroids, mycophenolate mofetil and calcineurin inhibitors. These tumours included 20 SCCs with subsequent local recurrences and 14 with subsequent metastases (10 to the lymph nodes, six in-transit and four distant ones; some SCCs developed metastases to more than one site). The primary A-SCCs were located on the head and neck (61%), the limbs (upper, 18%, lower 15%) and the trunk (6%). C-SCCs studied in parallel included 25 SCCs matched for body site, obtained from OTRs who had A-SCCs; they had been excised within six months (before or after) from excision of the A-SCC and had not developed recurrences or metastases over the same follow-up period.

Table I. *Pathological features of aggressive (A-SCC) and control (C-SCC) squamous -cell carcinomas in organ-transplant recipients*.

Feature		A-SCC (n=34)	C-SCC (n=25)	*p*-Value
Size, macroscopic (mm)		7.37±5.3	8.96±3.4	0.08
Ulceration		16 (47%)	12 (48%)	0.94*
Microscopic tumour thickness (mm)		4.0±3.5	2.8±1.7	0.39
Clark level**	II	2 (6.5%)	2 (8%)	0.03
	III	11 (35.5%)	17 (68%)	
	IV	9 (29%)	5 (20%)	
	V	9 (29%)	1 (4%)	
Deep tissue invasion		5 (15%)	0 (0%)	0.06
Acantholysis		13 (38%)	9 (36%)	1
Differentiation	Good	15 (44%)	16 (64%)	0.4
	Moderate	14 (41%)	7 (28%)	
	Poor	5 (15%)	2 (8%)	
Lymphovascular invasion		3 (9%)	0 (0%)	0.25
Perineural invasion		5 (15%)	0 (0%)	0.07
Peritumour infiltrate density	Dense	16 (47%)	13 (52%)	0.8
	Moderate	15 (44%)	11 (44%)	
	Absent/sparse	3 (9%)	1 (4%)	
Mitoses/5 HPF	Total	6.4±5.4	4.4±3.2	
Atypical		3.8±3.2	2.7±2	0.13

For numeric variables, *p*-values were obtained with the Wilcoxon test. For nominal variables, *p*-values were obtained with the Fisher's exact test (or *χ^2 test). **Data missing for three cases of A-SCC. HPF: High power fields.

The results of the pathological study are presented in detail in Table I. Briefly, when compared with C-SCCs, A-SCCs were similar as regards macroscopic size, ulceration, acantholysis and peritumoural infiltrate density, but had higher Clark levels and rates of deep tissue invasion, lymphovascular and perineural invasion, higher mitotic rate (both total and atypical) and lower degrees of differentiation. Among these features, the distribution of Clark levels proved statistically significant (p=0.03), as 58% (18/34) of A-SCCs were of Clark level IV or V *vs*. 24% (6/25) in the C-SCC group. Differences in the rate of deep tissue invasion and perineural invasion failed to reach statistical significance (p=0.06 and 0.07, respectively).

Discussion

Several previous studies have assessed the pathological features of aggressiveness of SCC in the population at large, but so far no study has specifically examined SCCs developing in OTRs, even though some of the previous studies included a (usually small) number of tumours from immunosuppressed patients, including OTRs (6).

Our results show that the depth of SCC invasion, as defined by the Clark level, is a significant factor predicting an aggressive outcome of SCC in OTRs. Indeed, 58% of A-SCCs were of Clark level IV or V contrasting with 24% in C-SCCs. They also suggest that compared with C-SCCs, A-SCCs tend to be thicker, less differentiated, and to have higher rates of mitosis, lymphovascular and perineural invasion and deep tissue invasion, which is not unexpected given the positive relation between the level of invasion (Clark) on the one hand, and deep tissue invasion and microscopic thickness on the other. On the other hand, our results do not support the contention that acantholysis is an adverse pathological feature (13), as this change was found at almost identical frequencies in A-SCC and C-SCC.

Overall, our present findings are in keeping with previous data concerning SCC in the population at large (4-12). The fact that statistical significance was reached only for differences in Clark levels is probably due to the small number of tumours included in this study, which is underpowered for confirming numerically small or modest differences. This limitation, *i.e.* the small number of tumours included in this study, is due to the stringent criteria we applied to the selection of patients and tumours, namely the fact that the former should have developed within a period of six months both aggressive and non-aggressive SCC, which implies a sufficient patient follow-up, and the availability of the initial pathological excision material (some of our patients had undergone excision of the primary SCC at other health centres and material was therefore unavailable for study). Conversely, our study is in other respects original. It was based on a blinded re-examination of pathological slides by two (dermato)pathologists using homogeneous predefined criteria for all SCCs studied, whereas most previous studies have retrieved retrospective data from pathology reports (which may be heterogeneous according to the laboratory and the pathologist examining the tissue sections). More importantly, as far as we are aware, our study is the first to have used 'internal' controls for the A-SCCs studied, *i.e.* SCCs from the same patients developing over the same time period, but that showed no aggressive behaviour during follow-up. This minimizes differences between the two SCC groups studied with respect to several factors possibly influencing tumour outcome in the transplant setting, such as patient gender and age, immunosuppression regimen and duration, the organ grafted and the level of patient surveillance. Furthermore, the matching of C-SCC to A-SCC as to tumour localisation minimized the influence of this factor, as it is known that tumour outcome varies depending on the anatomical location of the lesion.

The possibility of early (*i.e.* at the time of initial SCC excision) prediction of which SCCs are at higher risk for developing local recurrences and metastases is important, especially in OTRs, as these patients should be followed up closer after excision of a potentially aggressive tumour. Our study shows that a high (≥ IV) level of tumour invasion, *i.e.* including at least the whole dermis, is associated with an aggressive outcome of SCC in OTRs, therefore patients with such tumours should be carefully monitored, independently of other risk factors. Examination of a larger number of A-SCCs from OTRs will hopefully allow: a) confirmation of whether perineural, lymphovascular and deep tissue invasion (which tended towards significance in this study) do have a prognostic significance in the transplant setting as they do in the general population; b) whether additional pathological predictors of negative outcome exist; and c) which, if any, features are more specifically associated with recurrence and metastasis (the present study was underpowered to reveal such differences), and more importantly, with the risk of tumour-specific mortality. In this respect, the study of molecules associated with cell proliferation or oncogene expression (such as Ki67, p53 and p16) will hopefully also provide additional predictive information useful for tumour risk evaluation and thereby for patient management.

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