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CLINICAL REPORT

Correlation Between Histological Findings on Punch Biopsy Specimens and Subsequent Excision Specimens in Cutaneous Squamous Cell Carcinoma

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Diagnosis and subsequent treatment of cutaneous squamous cell carcinoma are frequently based on punch biopsies. Regarding the current TNM classification and stage grouping for cutaneous squamous cell carcinoma, it is important to identify the high-risk features (infiltration depth >4 mm, perineural and/or lymphovascular invasion and poor differentiation). This study investigates the agreement of histological high-risk features and TNM grouping stage on 3 mm punch biopsies and subsequent surgical excision in 105 patients diagnosed with cutaneous squamous cell carcinoma. On punch biopsy, infiltration depth >4 mm is not identified in 83.3% (30/36), perineural invasion in 90.9% (10/11) and poor differentiation in 85.7% (6/7) of cases. The TNM stage was underestimated on punch biopsy in 15.4% (16/104). This study shows that on a 3-mm punch biopsy, high-risk features in cutaneous squamous cell carcinoma can remain undetected and that the actual TNM stage is not identified in 1 out of 6 tumours. *Key words: squamous cell carcinoma; treatment; diagnosis; punch biopsy; surgical excision; TNM grouping stage.*

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Non-melanoma skin cancer is the most common cancer in Caucasians and constitutes more than one third of all cancers in the United States (1). Cutaneous squamous cell carcinoma (cSCC) is the second most common type of skin cancer, with an incidence of 20.5/100,000 persons/year in the Netherlands (1–3) and the incidence of cSCC is increasing by 3–8% per year (1, 4–7). cSCC has the ability to infiltrate local tissue, spread to regional lymph nodes in 3–8% or cause distant metastasis in 4–5% of patients (6, 8–10).

Previous studies showed that clinical examination of cSCC results in failure to correctly diagnose cSCC in 32% and that sensitivity is as low as 41.1% (11, 12). Therefore diagnosis of cSCC is mostly based on the histological findings of a punch biopsy. In the latest TNM classification and staging for cSCC, in addition to tumour diameter, it is important to identify high-risk features (Table I). T1 tumours with 2 or more high-risk features are considered stage II tumours. High-risk tumour features are infiltration depth greater than 4 mm, presence of perineural and/or lymphovascular invasion, poor differentiation and localisation on ear or lip (13). These tumour features are associated with an elevated risk of metastasis and local recurrence (13). National guidelines of the United States, United Kingdom, Australia, France and The Netherlands recommend a therapeutic surgical excision with a 4–6 mm safety margin for stage I tumours (14–18). Stage II–IV tumours are treated with an excision with 6–10 mm safety margin, Mohs micrographic surgery or radiotherapy (14–18). In patients with high-risk tumours, ultrasound of regional lymph nodes is advised, in diagnostic and/or follow-up period, with cytological or histological examination of enlarged lymph nodes (14, 16, 18). Accurate identification of the high-risk features and TNM grouping stage is important to determine the optimal treatment regimen (9, 16, 19, 20).

In contrast to basal cell carcinoma (BCC), the correlation between the histological findings of punch biopsies and surgical excision in cSCC has not been documented in the literature (21–24). In cSCC, not only the correla-

Table I. *TNM stage grouping in cutaneous squamous cell carcinoma (13)*

TNM stage	Tumour	Regional lymph nodes	Distant metastases
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1–3	N1	M0
Stage IV	T1–3	N2–3	M0
	T4	Any N	M0
	Any T	Any N	M1

*AJCC considers stage I tumours with more than one high-risk features as stage II.

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tion of histological differentiation is of interest, but also the high-risk features mentioned above.

The main purpose of this study was to establish the value of a punch biopsy for correctly identifying key prognostic factors in cSCC (infiltration depth > 4 mm, degree of differentiation, perineural invasion and lymphovascular invasion). In addition, we compared the TNM staging in biopsy and subsequent therapeutic surgical excision and investigated how often a cSCC was misclassified.

METHODS

A retrospective analysis based on histological findings of punch biopsy and the following surgical excision in primary cSCC was conducted. Histologically confirmed primary cSCC were derived from the Comprehensive Cancer Centre the Netherlands, region mid and South Limburg, The Netherlands. All patients diagnosed between January 1st 2005 and December 31st 2007 at the Maastricht University Medical Centre (MUMC) were included. In case a patient had multiple cSCCs, only the first cSCC was included. The cSCC had to be present on both punch biopsy and surgical excision specimens. Patients with a cSCC *in situ* were excluded. Patient and tumour characteristics were obtained from patient files at the MUMC. Histological slides were obtained from the archives of The Maastricht Pathology Tissue Collection. Collection, storage and use of these slides were performed in accordance to the "Code for Proper Secondary Use of Human Tissue in the Netherlands".

Procedure

Skin biopsies included 3 mm punch samples. Therapeutic surgical excision samples included specimens with a standard safety margin of 5 mm (for TNM stage I) and up to 10 mm for high-risk tumours (TNM stage II–IV). Mohs micrographic surgery and radiotherapy were performed in selected cases (24).

Histological examination

Primary diagnosis of cSCC was based on microscopic evaluation of histological slides of both the punch biopsy and the subsequent excision by dermatopathologists at our University Hospital. To avoid inter-observer variability, a retrospective assessment of all histological slides was then conducted by 2 investigators. A third year pathology resident (BL) and a sixth year medical student (MR), who received an intensive course in analysis of histological specimen of cSCC by an experienced dermatopathologist (AvM), examined all slides independently. In case of a different outcome, the slides were re-examined by both investigators in order to reach consensus. A randomly selected part of the slides was also judged by this dermatopathologist.

The primary tumour was evaluated in histological slides of both punch biopsy and subsequent surgical excision based on the following criteria: cSCC infiltration depth in mm, invasion of deeper structures (muscle, cartilage and bone), degree of differentiation (good, moderate, poor) (25), perineural invasion, lymphovascular invasion and TNM stage based on the 2009 TNM classification (13).

Statistical analysis

Distributions of patient characteristics, clinical tumour aspects and histopathological data are described as means with standard deviations or as proportions and absolute numbers. Tumour specific information regarding key prognostic factors of cSCC and TNM stage are compared in biopsy and excision specimen. The Pearson's correlation coefficient and explained variance are calculated for comparison of absolute infiltration depth. Cross tabulations and Cohen's Kappa (κ) statistics are performed to determine the degree of agreement for infiltration depth (≤ 4 mm versus > 4 mm), perineural invasion, lymphovascular invasion and degree of differentiation and TNM grouping stage. Subgroup analysis is performed for the cases where the bottom of the biopsy did not contain tumour. All data analyses were performed with SPSS (Statistical Package for Social Sciences) version 20.0.

RESULTS

Patient and tumour characteristics

The database revealed 224 patients with a histologically proven cSCC. A preoperative biopsy was taken in 71% of the cases. The patient and tumour characteristics were not significantly different in the patients with ($n = 152$) or without ($n = 72$) a preoperative biopsy. Of the 224 patients, 86 were excluded because the punch biopsy ($n = 73$) or the surgical excision specimen ($n = 13$) was not available, while 33 patients were excluded because there was no remaining carcinoma in the excision specimen. Therefore, 105 patients with 105 cSCCs were included. Out of these 105 patients, 67 were male and 38 were female. The mean age was 71.6 years. Tumour characteristics are shown in Table II.

Comparison of biopsy and excision results

Table III shows an overview of comparison on high-risk features in biopsy and therapeutic surgical excision specimen.

Infiltration depth

Comparison of the infiltration depth (in mm) of biopsy and subsequent excision specimen showed a Pearson's correlation coefficient (R) of 0.51 ($p < 0.01$). The explained variance (R^2) showed that 26% of the excision results for infiltration depth can be predicted by the biopsy results (Fig. 1). Furthermore a comparison of the infiltration subdivided into 2 categories, ≤ 4 mm versus > 4 mm, showed a corresponding assessment in 70.5% ($n = 74$) of the biopsies. In 28.6% ($n = 30$) the biopsy misclassified the tumour infiltration as smaller than 4 mm (Table III). In 59 of 105 punch biopsies, the tumour was present in the bottom of the biopsy.

In 46 patients in whom the bottom of the biopsy did not contain tumour, a subgroup analysis was performed. Tumour localisation and tumour diameter in this subpopulation were comparable to the overall study population. The mean tumour depth in the biopsy was 2.05 mm compared to 2.56 mm in the excision specimen. The depth ≤ or > 4 mm was not corresponding in 5 (10.9%) cases between biopsy and excision specimen.

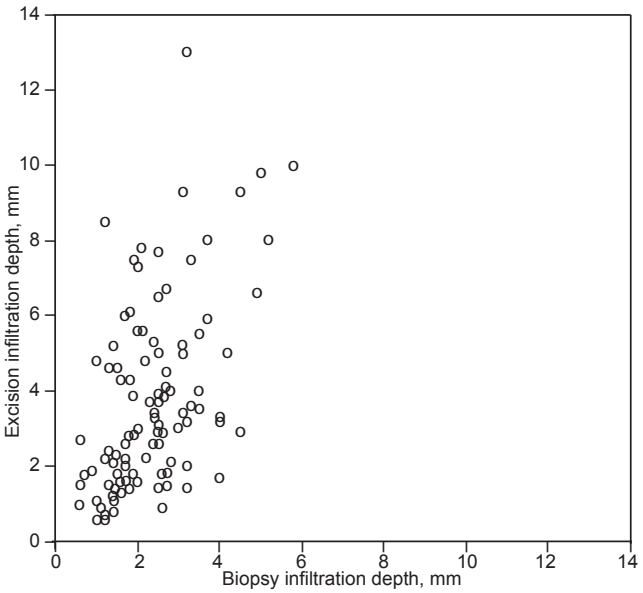

Fig. 1. Correlation between infiltration depth in mm between biopsy and excision in cutaneous squamous cell carcinoma. Pearson's correlation coefficient R=0.51.

Table II. *Tumour characteristics (n=105)*

Characteristics	*n* (%)
Tumour localisation	
Ear	18 (17.1)
Lip	9 (8.6)
Head/neck	50 (47.6)
Trunk	6 (5.7)
Arms	14 (13.3)
Legs	8 (7.6)
Tumour diameter	
≥2 cm	20 (19.0)
<2 cm	84 (80)
Unknown	1 (1.0)
Invasion in deeper structures	
Muscle	5 (4.8)
Cartilage	5 (4.8)
Muscle and cartilage	1 (1.0)
None	94 (89.5)
Degree of differentiation	
Well	27 (25.7)
Moderate	71 (67.6)
Poor	7 (6.7)
Perineural invasion	
Present	10 (9.5)
Absent	95 (90.5)
Lymphovascular invasion	
Present	1 (1.0)
Absent	104 (99.0)
TNM grouping stage	
I	70 (67.3)
II	21 (20.2)
III	13 (12.5)
IV	0 (0)
Unknown	1 (1.0)

Histological data are derived from the excision specimen.

Perineural invasion

In 89.5% ($n=94$) of the cSCCs, perineural invasion was absent in both punch biopsy and surgical excision. In the 10 cases (9.5%) where there was perineural invasion in the excision specimen, no perineural invasion was determined in the biopsy. In one case the biopsy revealed perineural invasion that could not be identified in the excision (Table III).

Lymphovascular invasion

Angio-invasion was absent in both biopsy and excision in 99% ($n=104$) of the cases. In the one case with angio-invasion in the excision specimen, the biopsy missed the angio-invasion. Cohen's Kappa statistics could not be computed for lymphovascular invasion because the variable is constant in all biopsies (Table III).

Degree of histological differentiation

In 71.5% ($n=75$) of the tumours, punch biopsy and surgical excision revealed the same degree of differentiation. Of the 7 poorly differentiated cSCCs in excision, 85.7% ($n=6$) were defined as moderately differentiated cSCC on punch biopsy (Table III). In addition, 16.9% ($n=12$) of the cSCCs were moderately differentiated on punch biopsy, of these 2.8% ($n=2$) were diagnosed as being poorly differentiated and 9.5% ($n=10$) as being well differentiated on excision.

TNM staging

In 87 (83.7%) cases, punch biopsy and surgical excision revealed the same TNM grouping stage. The use of a punch biopsy to determine the TNM staging in cSCC results in under-staging in 15.4% ($n=16$). Of all stage II and III tumours, 32.4% (11/34) was defined on punch biopsy as a stage I tumour. One stage I tumour in the excision was classified as stage II on punch biopsy (Table IV). All misstaged tumours were located in the head and neck region. Of all cSCCs on lip or ear, misstaging occurred in 48% (13/27) of the cases. In the subgroup of 46 patients in which the bottom of the biopsy did not contain tumour, 9% ($n=4$) of

Table III. *Comparison of high-risk features in biopsy and excision specimen in cutaneous squamous cell carcinoma*

	Observed agreement	High risk features not identified on punch biopsy	Kappa coefficient
Infiltration depth (≤ 4 mm vs. > 4 mm)	70.5% (74/105)	83.3% (30/36)	0.188
Perineural invasion (present versus absent)	89.5% (94/105)	90.9% (10/11)	–0.180
Lymphovascular invasion (present versus absent)	99% (104/105)	100% (1/1)	–
Histological differentiation (poor versus moderate/well)	71.5% (75/105)	85.7% (6/7)	0.352

Table IV. *TNM grouping stage in biopsy and excision (*n = *104*)*

	Excision				
Biopsy	I *n* (%)	II *n* (%)	III *n* (%)	IV *n* (%)	Total *n* (%)
I	69 (66.3)	5 (4.8)	6 (5.8)	0 (0)	80 (76.9)
II	1 (1)	16 (15.4)	5 (4.8)	0 (0)	22 (21.1)
III	0 (0)	0 (0)	2 (1.9)	0 (0)	2 (1.9)
IV	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	70 (67.3)	21 (20.2)	13 (12.5)	0 (0)	104 (100)

*One case is missing because of unknown tumour diameter.

the TNM grouping stage was different between biopsy and excision specimen.

Interobserver agreement

A third observer (dermatopathologist) judged 22% of all histopathological slides, which were randomly selected. The interobserver agreement was substantial to excellent for the following factors: tumour thickness Kappa 0.91, tumour differentiation kappa 0.65 and perineural invasion kappa 0.64. Cohen's Kappa statistics could not be computed for lymphovascular invasion due to low incidence.

DISCUSSION

Our results showed that high-risk features in cSCC can remain undetected on 3 mm punch biopsies. Furthermore, the actual TNM grouping stage is not identified in 1 out of 6 tumours. These results indicate the importance of establishing a definite TNM staging after the therapeutic excision.

Comparison of the infiltration depth of biopsy and subsequent excision showed a substantial disagreement. A punch biopsy may not be trusted to predict an accurate infiltration depth. Part of the difference may be explained by a time delay between biopsy and excision, in which progression of infiltration depth may occur. In 59 of 105 punch biopsies, the tumour was present in the bottom of the biopsy. In these cases the punch biopsy is not deep enough to determine the infiltration depth accurately. We therefore conducted a subgroup analysis and found that in cases where the tumour was not present in the bottom of the biopsy, the reported infiltration depth was more reliable. It is important that all physicians who perform punch biopsies for diagnosis have to be trained in taking deep biopsies. Furthermore, physicians should be aware of the limitation in depth of 3 mm punch biopsies. In cases where the tumour is located at the bottom of the biopsy, this should be mentioned in the pathology report and depth should best be regarded as 4 mm or more. A study by de Visscher et al. (26) regarding the relationship between tumour thickness in punch biopsy and surgical excision of SCC of the lip showed a tumour thickness within 0.5 mm in 55.5% of patients. Furthermore the study showed that a 2–4 mm punch biopsy is only useful for tumours less than 3 mm thick. For a tumour thicker than 3 mm a better agreement in tumour thickness may be obtained by a larger incision biopsy rather than by punch biopsy (26).

Clinically, 80% of the tumours were smaller than 2 cm. Determining the high-risk factors for these patients can make the difference between TNM grouping stage I and II (13–18). Stage III tumours have invasion in deeper structures, for example muscle or bone. In most cases biopsies will not reveal this deeper invasion, as biopsies are generally not taken beyond the subcutaneous fat tissue. Stage IV tumours, i.e. tumours invading the axial skeletal, were not found in the study population. The overall agreement of TNM staging in our study between punch biopsy and excision was 83.7%. In 15.4% of the patients the TNM grouping stage was underestimated by punch biopsy. It is well known that localisation of a cSCC on the lip or ear is a risk factor for metastasis (27, 28). In our study all understaging occurred in head and neck cSCC. Of all tumours on the ear or lip, 48% were misstaged on punch biopsy.

Hollestein et al. (3) showed differences in 5-year survival rates for the TNM stages. The 5-year survival rate for stage I tumours is 95%, compared to 76% for stage II and 62% for stage III/IV tumours. Misstaging has implications for patient management, as a higher TNM stage requires a more aggressive approach. A broader excision and more diagnostic tests, like an ultrasound of regional lymph nodes, might be necessary (14–18). Also, a time delay in searching for metastatic disease and definite treatment can occur.

A number of limitations of this study have to be taken into consideration. The study design was retrospective and not all slides could be retrieved. Furthermore, the high-risk feature lymphovascular invasion could not be analysed because only one case was found in excision specimen and none in biopsy specimen. A study on metastatic cSCC and its risk factors in 8,997 patients showed an incidence of 1% lymphovascular invasion, implying that the incidence of lymphovascular invasion in our population was representative for the average population of patients with cSCC (27). Poor differentiation and perineural invasion were analysed but the number of tumours with these features was relatively low. A strong feature of this study is that all histological slides were re-examined by the same observers excluding interobserver variability of assessment.

A punch biopsy is not completely reliable for histological subtyping cSCC. New techniques might raise this reliability. A few molecular markers are described in the literature with different expression in cSCC subtypes. Phosphorylated STAT3, a signal transducer and activator of transcription marker, has a stronger expression in poorly differentiated cSCC, while in poorly differentiated tumours there is an increased proliferation rate (Ki-67 index) (29, 30). Depth of invasion was associated with phosphorylated STAT3 and MMP-1 expression in stromal cells (29, 31). Other investigated molecular markers,

for example p53, cyclin D1 and E-cadherin, do not seem to add to subtyping cSCC as they have comparable expression in well and poorly differentiated cSCC (30, 31).

This is the first study on correlation of high-risk features and TNM grouping stage of 3 mm punch biopsy and subsequent surgical excision in cSCC. Substantial differences were found in histopathology between punch biopsy and the subsequent surgical excision specimen. In 1 out of 6 tumours, the TNM grouping stage had to be revised after surgical excision. Of the tumours located on the lip and ear 48% is misclassified. In conclusion, punch biopsies are adequate in the diagnosis cSCC. However, the high-risk features, particularly in head and neck tumours, are less reliable on punch biopsy. Therefore, definite TNM staging has to occur after surgical excision, especially in head and neck region. Further research should reveal if taking larger punch or incision biopsies and/or new molecular techniques lead to more accurate staging of cSCC on biopsy.

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