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INVESTIGATIVE REPORT

Pre-treatment Evaluation of Basal Cell Carcinoma for Photodynamic Therapy: Comparative Measurement of Tumour Thickness in Punch Biopsy and Excision Specimens

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Tumour thickness affects the outcome of photodynamic therapy in basal cell carcinoma (BCC). The aim of this study was to evaluate whether punch biopsy provides reliable information on BCC tumour thickness, by comparing corresponding measurements in biopsy and excision specimens for 48 lesions in 43 patients. BCC tumours were between 0.2 and 6.1 mm thick. The mean depth of the excisions were 0.14 mm greater than that of the biopsies. Bland-Altman 95% limits of agreement were (–1.3, 1.6) mm, but the difference between measurements increased with tumour thickness. A punch biopsy tumour thickness of 1.0 mm yielded an upper 95% predicted limit for excision depth within 2.0 mm. In conclusion, there was reasonable overall agreement between corresponding measurements. A biopsy thickness of 1.0 mm suggests that the tumour will most likely be within the current accepted limits for photodynamic therapy. With increasing tumour thickness, however, individual tumour measurements may differ considerably. ***Key words: skin cancer; basal cell carcinoma; tumour thickness; biopsy punch; microscopic measurement; topical photodynamic therapy.***

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Basal cell carcinoma (BCC) is the most common type of skin cancer in the adult, white population and has an increasing incidence worldwide (1, 2). It is a slow-growing tumour that can show infiltrative irregular extensions and outgrowths on histological examination (3, 4). Despite its low metastatic potential, this tumour can cause significant local tissue destruction and patient morbidity (5). Given that BCC has a predilection for sun-exposed skin on the head, face and neck, cosmetic outcome may be important when choosing therapy (6, 7).

Topical photodynamic therapy (PDT) has shown cosmetic superiority over traditional therapies including surgical excision (8, 9). It involves the accumulation of a photosensitizer in neoplastic tissue, which is then activated by red light, thus inducing a photochemical reaction that results in tissue destruction (10, 11).

Tumour thickness is an important predictor of metastasis in malignant melanoma (12) and squamous cell carcinoma (SCC) (13, 14); in BCC it may affect the response to PDT (15–19) since this therapy has limited skin-penetrating abilities (20). The photosensitizers most commonly in use have been shown to penetrate BCC tumours efficiently to a depth of only approximately 2.0 mm (20–22), and red light also has a limited penetration into tissue (23). Several studies have demonstrated lower PDT response rates in nodular tumours compared with superficial lesions (15, 17, 23–25). Therefore, current guidelines preferentially recommend the use of topical PDT for thin lesions (10, 26), and hence support the need for reliable pre-treatment assessment of BCC tumour thickness (27).

In addition to an accurate diagnosis, a biopsy specimen for histopathological examination provides information about the depth of tumour invasion and the histological growth pattern (28–30).

Punch biopsy is widely used and is generally considered the primary technique to obtain full-thickness diagnostic tissue (31). With this technique tissue architecture is well preserved, and even small diameter samples provide material of sufficient size and quality for reliable histological diagnosis (32). However, a biopsy punch will offer information from only a restricted selected tumour area compared with an excision specimen, which allows more extensive examination of a lesion. One may thus question the ability of a single biopsy reliably to reflect tumour depth in a given lesion. It is therefore of clinical and scientific interest as to whether biopsy measurement of tumour thickness is an accurate basis for treatment planning and apposite as a reference in research work.

The aim of this prospective study was to evaluate whether punch biopsies provide reliable information about BCC tumour thickness, by investigating the agreement between measurements made on punch biopsy and excisional specimens from the same lesions.

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MATERIALS AND METHODS

Patients referred to the outpatient clinic at the Department of Dermatology, St Olav's University Hospital, Trondheim, with primary tumours clinically suggestive of BCC and suitable for excision surgery were assessed for eligibility. Exclusion criteria were: age less than 18 years; pregnancy or breastfeeding; lesions with a clinically largest diameter less than 9.0 mm; lesions in which excision surgery by a plastic surgeon was the treatment of choice.

Lesion sizes were clinically defined as the mean of the length and width measurement. Local anaesthesia, using lidocaine 1% with adrenaline, was infiltrated intradermally before taking of samples. A sterile, steel, disposable biopsy punch (Kai Industries Co. Ltd, Gifu, Japan) 3 mm in diameter, was used in all cases. One punch biopsy was obtained from each lesion prior to the surgical excision of the same lesion. Three dermatologists performed this procedure, each on 24, 19 and 12 lesions, respectively.

The biopsy punch was taken from the part of the tumour that was clinically considered to be thickest by inspection and palpation. If the tumour appeared to be homogeneous, the biopsy was taken from the central area. After obtaining the punch biopsy, the whole tumour was excised using a full-thickness ellipse resection. Corresponding punch biopsy and excisional specimens were obtained in all cases, and examined by one hospital pathologist.

The depth of the punch biopsy tissue was routinely measured after fixation in formaldehyde. The tissue was further subjected to a dehydration process by immersion in increasing concentrations of ethanol, clearance in xylene and, finally, casting in paraffin wax to make it stable and easy to cut with a microtome.

The punch biopsy was oriented so that the epidermis aligned with the longest axis of the wax block. Three parallel, interspersed sections were cut out of the block. The sections were stained with haematoxylin, eosin and saffron (HES) and examined under a microscope. The thickness of the tumour was measured from below the stratum corneum to the bottom of the tumour nest. Tumour thickness and investigation of disease-free deep margin, defined as at least 0.1 mm of tumour-free tissue, were based on measurements using an ocular micrometer (Pierre Verniers method) to a precision of 0.1 mm (28). The largest measurement of the three histologically prepared sections from each punch biopsy specimen was defined as the punch tumour thickness. The surgically removed excision specimen was oriented by an attached suture before being cut into 3–8 slices in accordance with the breadloaf sectioning method (33). The number of slices was dictated by specimen size, each with a thickness of 2–3 mm. Following the same procedure as for punch biopsy, the 3–8 slices were processed and cast into 2–3 blocks of paraffin wax. Sections representative of both central and peripheral areas of the lesion were cut from the blocks. Assessment of tumour thickness and investigation of disease-free surgical margins were carried out as described for the punch biopsy specimen. The largest measurement obtained in the histologically prepared sections from a surgical excision specimen was defined as the excisional tumour thickness.

The excisional specimens were histologically subclassified into three categories; superficial, nodular, and aggressive-growth types. The last category included the morpheiform, infiltrative and basosquamous types (34).

The study was approved by the local ethics committee (REK number 4.2007.558) and patients provided written informed consent prior to study entry.

Statistical methods

The agreement between punch biopsy and excision specimen tumour thickness measurements was investigated in several ways. First, the mean difference (i.e. bias) between methods was analysed using a paired samples *t*-test. Secondly, each method of measurement was plotted against the other, and their difference against the mean in a Bland-Altman plot (35). This plot allows a visual expression of how well the two methods agree across the range of measurements, and provides 95% limits of agreement. Finally, as punch biopsy will always precede excision of the tumour, we obtained 95% prediction limits for excision tumour thickness given punch biopsy measurements employing the regression approach described by Carstensen (36). This method can take an increasing standard deviation into account.

Different lesions from the same patient were considered to be independent, and the statistical software R (37) 2.11.1 was employed for all analyses.

RESULTS

Fifty patients, with a total of 55 lesions clinically suggestive of BCC, were initially included in the study. On histological examination, five lesions proved to represent actinic keratosis or SCC, lymphoma in one case, and a benign naevus in one case. These seven lesions were excluded; thus 48 lesions from 43 patients (21 women with 23 lesions, 22 men with 25 lesions) were included. Mean patient age at presentation was 74 years (range 47–97). Thirty-nine patients presented with one lesion, three had two lesions and one had three lesions. Most lesions ($n = 28$) were located on the trunk. The remainder were located in the head and neck region ($n = 14$), or on the extremities ($n = 6$). Mean lesion size was 11.59 mm (range 7.5–18.0 mm). Histologically, 19 tumours were of superficial, 18 of nodular and 11 of aggressive-growth type. The length of the biopsy specimens ranged from 2.0 to 11.0 mm, with a mean length of 5.3 mm.

Tumour thickness could not be determined with certainty in three biopsy and two excisional specimens from five different lesions, as tumour tissue was observed within the deepest part of the histologically prepared sections. In these cases a measurement was taken from below the stratum corneum to the lower part of the section.

The mean punch tumour thickness was 1.53 mm (range 0.2–5.2 mm), and mean excisional tumour thickness was 1.67 mm (range 0.3–6.1 mm); yielding a mean difference between measurements (i.e. the bias) of 0.14 mm.

We found identical (within 0.1 mm) measurements of tumour thickness using the two methods in 7 lesions. Surgical excision gave the largest measurement in 23 (55%) of 41 specimens. For tumours less than 2.0 mm thick, surgical excisions gave the largest measurement in 61% of cases. For tumours equal to or thicker than 2.0 mm either method yielded the largest measurement.

Fig. 1 shows the Bland-Altman plot with 95% limits of agreement (–1.33 mm to 1.6 mm) for the difference between the two measurements. The plot shows a widening scatter as the average thickness increases, i.e. an increasing disparity between the two methods.

Figs 1 and 2 clearly demonstrate that the difference between measurements increases as tumour thickness increases; this was confirmed in the regression analysis ($p < 0.01$). Fig. 2 shows the scatter plot of punch biopsy vs. excision specimen measurements, including the upper 95% limit of prediction.

Interpretation of Fig. 2 is as follows: with a punch biopsy tumour thickness of 1.0 mm, the corresponding excision tumour thickness will, with 95% probability, be less than approximately 2.0 mm. With a punch biopsy of 2.0 mm, the limit is 3.5 mm; and with biopsy measurements beyond 2.0 mm the limits diverges strongly.

DISCUSSION

The accuracy of pre-treatment tumour thickness assessment in BCC is of importance to ensure an adequate selection of lesions suitable for PDT.

In the present study, we have described and quantified the agreement between corresponding measurements of BCC tumour thickness in punch biopsy and surgical excision specimens.

The mean depth of the excisions were 0.14 mm greater than that of the biopsies. The disparity between the two methods, however, increased with increasing tumour thickness.

The true extent of tumour thickness is unknown, as an accurate measurement of the thickest part of tumour is not always available. The surgical specimen cannot be regarded as a "gold standard", first because the biopsy punch may have removed the thickest tumour area. Secondly, in accordance with standard histopathological examination merely limited sections of the excision specimens were made, hence the risk of not detecting maximum tumour depth (33).

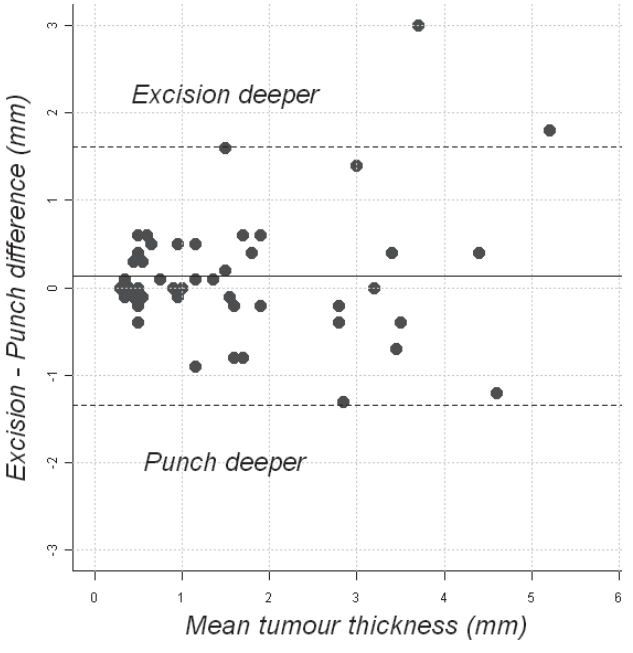

Fig. 1. Bland-Altman plot showing difference between measurements by excision and punch biopsy, according to mean tumour thickness. The mean difference (bias) was 0.14 mm, with 95% limits of agreement at –1.3 and 1.6 mm.

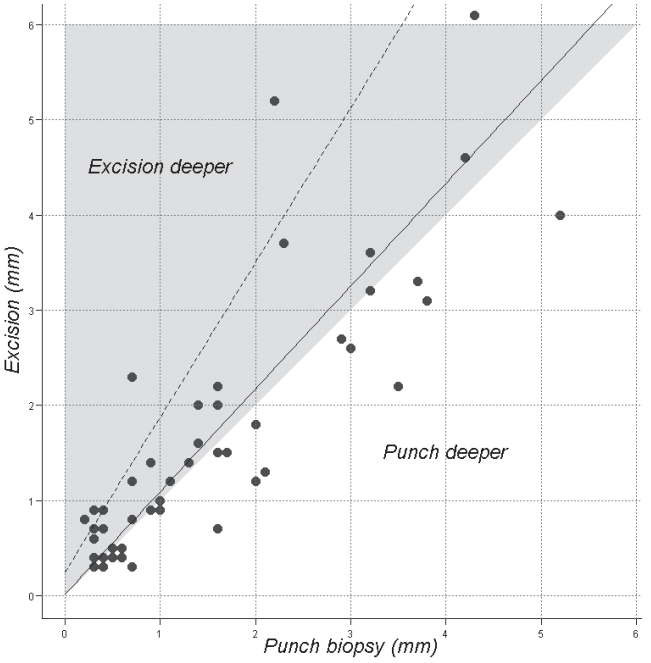

Fig. 2. Relationship between measurements of tumour thickness in corresponding punch biopsy and excision specimens. The shaded, grey area shows where excision depth exceeds punch biopsy depth with the line of identity at the border. The mean (——) and 95% upper (- - -) prediction lines are superimposed.

The clinical and histological diagnosis agreed in 87% of all biopsied lesions in this study. This is in line with previous studies that show the clinical diagnosis to be inferior to the histological diagnosis in BCC (30, 38). The punch biopsies were given priority, in the sense that they were taken prior to the excisions and from the thickest part of the tumour according to the clinical evaluation. Nevertheless, the largest tumour thickness was slightly more often found by surgical excision. Both techniques provided adequate tissue samples, including sufficient representative material for the investigation of deep tumour margins in almost all cases.

A variety of diagnostic technologies, such as optical coherence tomography and high-frequently ultrasound, are under investigation for non-invasive diagnosis of non-melanoma skin cancer (39). Recent studies have shown promising results with respect to the evaluation of tumour thickness of BCC lesions (16, 27). However, non-invasive imaging techniques are so far experimental with respect to evaluation of skin tumours, and biopsy specimens for histopathological examination of BCC are still considered to be the reference standard.

A variety of histopathological BCC subtypes are described and their global distribution shows a predominance of the nodular type, which most often appears on the head (40). In the present study, however, most

of the BCCs were of the superficial type and located on the trunk. It is possible that a number of patients with nodular facial tumours were primarily referred to the Department of Plastic Surgery, where advanced facial surgical procedures are performed at our hospital; consequently these patients would be unavailable for this study. Nodular tumours tend to grow deeper into cutaneous tissue than the superficial type (5, 40). With more nodular tumours included, lack of agreement might have been even worse.

Topical PDT is an effective treatment for superficial lesions and may also be considered in nodular lesions where alternative treatments such as surgery may be suboptimal (41).

The present international PDT consensus guideline (26) does not recommend treating BCC tumours that are thicker than 2.0 mm. The taking of pre-treatment biopsy samples for assessment of tumour thickness is encouraged (42), and is currently a supportive diagnostic method often used in clinical practice to provide information and select tumours suited for PDT.

However, two recent studies did not find any correlation between pre-treatment BCC thickness and PDT treatment failure (43, 44). The hypothesis in one of these studies was that thickness measurements from biopsy specimens might not be representative for tumour thickness of the entire lesion. This idea is supported by the findings in the present study, which question the ability of a single biopsy reliably to reflect tumour thickness. Even though a pre-treatment tumour thickness biopsy measurement of 1.0 mm suggests that the BCC tumour most likely will be within the current PDT consensus guideline recommendations of 2.0 mm, tumours that measure more than 1.0 mm on biopsy may well exceed this limit.

It should be noted, however, that the prediction interval presented in Fig. 2 derives from a limited number of observations.

The value of biopsy-based thickness measurements in BCC has, to our knowledge, not been presented previously. However, in a different type of non-melanoma skin cancer, a study of SCC of the lower lip (14), the relationship between biopsy with excision specimen tumour depths has been investigated. In this study a considerable disparity between corresponding measurements were found; in particular for tumours more than 3.0 mm thick. Together with the results from the present study, it appears that a single pre-treatment biopsy measurement in thick lesions may prove insufficient for selection of treatment, and this should be acknowledged when deciding on individual patient management and with regard to research in this field.

In conclusion, we found reasonable overall agreement between punch biopsy and surgical excision measurements of BCC tumour thickness when we compared the mean measurements of the two groups. A biopsy tumour thickness of 1.0 mm suggests that the tumour is likely to be within the current accepted limits for PDT. The paired measurements of individual lesions may, however, differ considerably, and this disparity increases with increasing tumour depth.

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Conflict of interest: Dr Christensen has received travel expenses connected to meetings and taken part in clinical trials with Photocure ASA, Oslo, Norway, but has not received any personal payment for this work. Dr Rørdam has a financial interest in Sklar Corporation and Personna Medical, both producers of surgical instruments. Dr Mjønes, Dr Foss and Professor Skogvoll have no conflict of interest to declare.

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