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Pre-treatment Assessment of Basal Cell Carcinoma for Topical Photodynamic Therapy and Long-term Treatment Outcome

Thesis for the degree of Philosophiae Doctor

Trondheim, April 2013

Norwegian University of Science and Technology
Faculty of Medicine
Department of Cancer Research and Molecular Medicine

NTNU – Trondheim
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ST. OLAVS HOSPITAL
TRONDHEIM UNIVERSITY HOSPITAL

NTNU
Norwegian University of Science and Technology

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ISBN 978-82-471-4298-1 (printed ver.)
ISBN 978-82-471-4299-8 (electronic ver.)
ISSN 1503-8181

Doctoral theses at NTNU, 2013:100

Printed by NTNU-trykk

Vurdering av basalcelle karsinom forut for lokal fotodynamisk terapi og langtidsresultat etter behandlingen

Avhandlingen er basert på studier som evaluerer diagnostiske metoder av basalcelle karsinom (BCC) forut for lokal fotodynamisk terapi (PDT) og langtidsresultat etter behandlingen. Vi fant godt samsvar mellom cytologisk og histologisk diagnostikk. Informasjon om BCC tykkelse fra stansebiopsier, kunne ikke alltid forutsi hvilke BCC som egnet seg for PDT. PDT av små, primære BCC gav få tilbakefall og et godt kosmetisk resultat 6 og 10 år etter behandling.

BCC er den vanligste form for kreft hos mennesker med lys hud og opptrer ofte på soleksponerte, kosmetisk utsatte hudområder som i ansiktet. Effektive diagnostiske og terapeutiske metoder, som bidrar til å gi et best mulig kosmetisk resultat, er således etterspurt. Prøvetakingen for cytologisk diagnostikk påfører huden minimal skade sammenlignet med biopsitaking for histologisk undersøkelse, og bør derfor vurderes. PDT er en vevsbesparende, minimal-invasiv behandlingsmetode som gir et godt kosmetisk resultat og som i økende grad brukes til behandling av BCC. Metoden baserer seg på at et lysfølsomt stoff akkumuleres hovedsaklig i de syke cellene. Ved belysning med synlig (rødt) lys og tilstedeværelse av oksygen dannes reaktive oksygen forbindelser som ødelegger de cellene som har høy konsentrasjon av det lysfølsomme stoffet. Tykkelsen av BCC kan påvirke behandlingsresultatet ved PDT. Både middelet (aminolevulinsyre, ALA) og lysstrålene som benyttes i behandlingen har begrenset evne til å penetrere huden. PDT er derfor i dag anbefalt brukt i behandling av tynne BCC (tykkelse < 2.0 mm). Pålitelig informasjon om BCC tykkelse bør derfor foreligge før PDT eventuelt velges som behandlingsmetode. Kurettering av BCC og bruk av dimethyl sulfoxide (DMSO), som øker penetrasjonen av ALA, kan bidra til å bedre behandlingseffekten av PDT. Kunnskap om langtidsresultater er nødvendig for evaluering av behandlingsmetoden.

I den første studien i avhandlingen ble cytologisk diagnose av ikke-melanom hudkreft (BCC og aktinske keratoser) sammenliknet med histologisk diagnose (”gullstandard”). Hos 41 pasienter med BCC ble 150 cytologiske utstryk (skrapeteknikk: n= 100, trykkteknikk: n= 50) undersøkt. Parvise prøver ble tatt fra samme lesjon til cytologisk og histologisk undersøkelse. Utstrykene ble farget enten med Papanicolaou eller May-Grünwald Giemsa metode. Skrapeteknikken viste 95 % samsvar med den histologisk diagnosen. Det var ingen vesentlig forskjell i resultatene mellom de to fargemetodene. Trykkteknikken gav mange utstryk med dårlig kvalitet og viste diagnostisk samsvar med histologi i 62 %.

I den andre studien ble 48 BCC hos 43 pasienter undersøkt. Vi sammenlignet mål på BCC tykkelse i parvise prøver fra individuelle lesjoner tatt med stanse- og eksisjonsbiopsi. Vi fant liten forskjell i gjennomsnittlig mål på BCC tykkelse i de to

gruppene (stansebiopsi1,53 mm, eksisjon 1,67 mm). Ved undersøkelse av enkeltstående tynne BCC var det et rimelig godt samsvar mellom de to metodene. BCC tykkelse < 1,0 mm, målt i en stansebiopsi, ville ved eksisjon mest sannsynlig gi en tykkelse som ligger innenfor gjeldende aksepterte grenser for PDT, på cirka 2,0 mm. I tykkere svulster fant vi større forskjeller mellom metodene.

I de to siste studiene ble 44 pasienter med 60 BCC behandlet en eller to ganger med kurettering og lokal DMSO-ALA-PDT, og deretter regelmessig kontrollert i henholdsvis 6 og 10 år. Av antall BCC klinisk vurdert som kurert 3 måneder etter behandling, forble 81 % sykdomsfrie etter 6 år; 91 % ved PDT gitt to ganger. Ved evaluering av alle BCC som fikk behandling, fant vi en helbredelses rate på 75 % etter 10 år; 90 % ved primær BCC og to PDT behandlinger. Ved kontroll ble BCC påvist i 15 behandlingsfelt; alle i løpet av de første 3 år etter PDT. Faktorer forbundet med behandlingssvikt var mannlig kjønn, BCC lokalisert sentralt i ansikt og på ører, residiv BCC (tilbakefall etter tidligere behandling) og kun én PDT behandling. Det kosmetiske langtidsresultatet ble vurdert som godt eller utmerket i alle tilfeller.

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Finansieringskilde: Samarbeidsorganet mellom Helse Midt-Norge RHF og NTNU

Overnevnte avhandling er funnet verdig til å forsvares offentlig for graden ph.d. i klinisk medisin.

Disputas finner sted i seminarromet, 1902-bygget, St. Olavs Hospital, Trondheim mandag 22.april 2013, kl 12.15.

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ACKNOWLEDGEMENTS

The present work emanates from the Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology (NTNU) and Department of Dermatology, St. Olav Hospital, Trondheim University Hospital, Norway. The work was funded by the Liaison Committee between the Central Norway Regional Health Authority (RHA) and NTNU. All studies took place at the Department of Dermatology, St. Olavs Hospital, Trondheim University Hospital.

First, I would like to express my gratitude to all patients who over the years have agreed to participate in the studies.

Then, I wish to express my warm thanks to my three supervisors. Professor Cato Mørk, Department of Cancer Research and Molecular Medicine, Faculty of Medicine, NTNU, who made the effort to be my principal supervisor in this project over the past three years. Your professional background of many years in dermatology has provided the project with a solid scientific platform. I thank you for technical advice, critical review of the work, swift feedback, helpful suggestions and a seemingly never-ending positive attitude. Associate professor Anna M. Bofin, Department of Laboratory Medicine, Children's and Women's Health, Faculty of Medicine, NTNU, has guided me and worked with me in various project over many years and has provided support and wise reviews. By means of your extensive experience, the use of cytology in the diagnosis of skin diseases has been highlighted. You took me along and showed me a universe of exciting shapes and beautiful colours. Your constructive suggestions and exciting ideas with regard to my present work and in relation to further development of parts of this project is highly appreciated. Professor and consultant Eirik Skogvoll, Department of Circulation and Medical Imaging, Faculty of Medicine, NTNU and Department of Anaesthesiology and Emergency Medicine, St. Olavs Hospital, Trondheim University Hospital, has guided me in this project long before it took the form of a doctoral dissertation and has not least contributed with invaluable statistical considerations and calculations. For someone who normally works in a field that holds life or death, it is admirable how you faithfully, for many years, patiently have followed me up to several millimetres into the skin. Apart from the purely academic, I would like

to thank you for always giving me constructive feed-back, joy and inspiration to continue on the way forward.

In addition, I want express my gratitude to dr. Stein Sundstrøm, who served as my principal supervisor the first period of this project.

Further, I would like to acknowledge the Department of Cancer Research and Molecular Medicine, NTNU, for constructive collaboration, and the head of the Department of Dermatology, St. Olav Hospital, Trondheim University Hospital, for incorporation of clinical research work in the department and for support of my PhD. I also want to thank my colleagues and additional personnel in the Department of Dermatology who have contributed to the implementation of this project and to thank my co-authors for their helpful contributions.

LIST OF PAPERS

This thesis is based on the following papers, referred to in the text by their Roman numerals:

I. Christensen E, Bofin A, Guðmundsdóttir, Skogvoll E. Cytological diagnosis of basal cell carcinoma and actinic keratosis, using Papanicolaou and May-Grünwald Giemsa stained cutaneous tissue smear. *Cytopathology* 2008; **19**: 316-22.

II. Christensen E, Mjønes P, Foss OA, Rørdam OM, Skogvoll E. Pre-treatment evaluation of basal cell carcinoma for photodynamic therapy: comparative measurements of tumour thickness in punch biopsy and excision specimens. *Acta Derm Venereol* 2011; **91**: 651-4.

III. Christensen E, Skogvoll E, Viset T, Warloe T, Sundstrøm S. Photodynamic therapy with 5-aminolaevulinic acid, dimethylsulfoxide and curettage in basal cell carcinoma: a 6-year clinical and histological follow-up. *J Eur Acad Dermatol Venereol* 2009; **23:** 58-66.

IV. Christensen E, Mørk C, Skogvoll E. High and sustained efficacy after two sessions of topical 5-aminolaevulinic acid photodynamic therapy for basal cell carcinoma: a prospective, clinical and histological 10-year follow-up study. *Br J Dermatol* 2012; **166**: 1342-8.

ABBREVIATIONS

AK	actinic keratosis
ALA	aminolaevulinic acid
BCC	basal cell carcinoma
CI	confidence interval
CM	confocal microscopy
DMSO	dimethylsulfoxide
EDTA	etylenediaminetetraacetic acid
HES	haematoxylin-eosin-saffron
HFUS	high-frequency ultrasound
LED	light emitting diode
DNA	deoxyribonucleic acid
MAL	methyl aminolaevulinate
MGG	May-Grünwald Giemsa
MMS	Mohs micrographic surgery
NMSC	non-melanoma skin cancer
OCT	optical coherence tomography
Pap	Papanicolaou
PDT	photodynamic therapy
PpIX	protoporphyrin IX
PTCH	patched gene
ROS	reactive oxygen species
SCC	squamous cell carcinoma
UVR	ultraviolet radiation

INTRODUCTION

The skin is the largest organ of the body and is divided into three main layers; the epidermis, dermis and subcutaneous tissue.[1] The epidermis is the outer part of the skin and is composed of multi-layered squamous epithelial cells (keratinocytes) and specialized dendritic cells (mainly Langerhans cells and melanocytes). In the lower part of the epidermis (stratum basale) cells divide and as they mature they proceed gradually towards the skin surface and eventually die **(Figure 1).** The outermost layer of the epidermis (stratum corneum) consists of cells without nuclei or organelles. Its structure provides an important barrier property of the skin that protects the body from infection, dehydration, chemical and mechanical assault. This barrier mainly consists of protein-enriched cells and of a lipid bilayer. The epidermis is separated from the dermis by a basement membrane, the primary function of which is to anchor the epithelium to the dermis. The dermis consists of connective tissue and is divided into the superficial papillary and the deeper reticular layers. It contains blood vessels, lymph vessels, hair follicles, eccrine and apocrine sweat glands, sebaceous glands and nerves. The subcutaneous layer is mostly composed of fat and connective tissue.

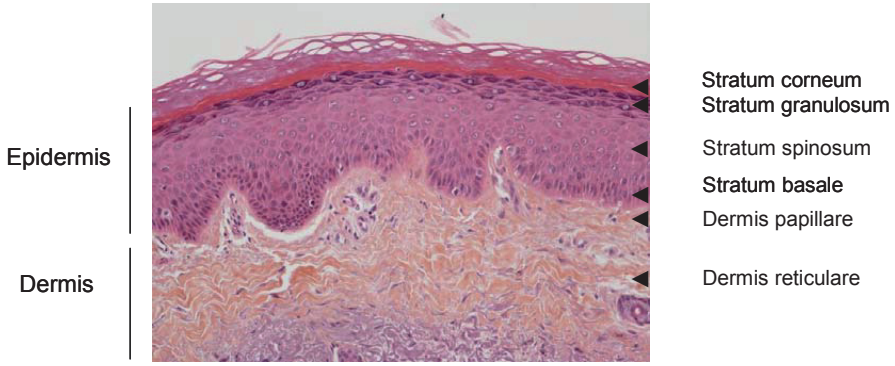


Figure 1.

Histological picture of normal skin. HES (x200). (Provided by Bofin AM.)

Skin damage can induce uncontrolled cell growth and skin cancer.

Basal cell carcinoma

The three major types of skin cancer are malignant melanoma, squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). BCC is the most common cancer in the fair-skinned population. It accounts for approximately 75% of all non-melanoma skin cancers (NMSC) and its incidence is increasing.[2] The annual incidence rates vary in different parts of the world with higher incidence in locations near the Equator. The rate is reported to be from 40 to 200 per 100 000 people per year in Northern Europe to over 700 per 100 000 people per year in Australia.[3,4] A total of 5 163 new BCC cases were reported to the Cancer Registry of Norway in 2008, after which time the Registry has discontinued registration of BCC in their reports (oral communication with the Cancer Registry of Norway). The true Norwegian numbers of new cases is most likely much higher, given that the population of Sweden is about twice that of Norway and that more than 30 000 BCCs are annually reported to the Swedish Cancer Registry.[5] Although the incidence of the disease increases with age, BCC is becoming more common in younger patients, especially women under 40 years old.[6] The life time risk of BCC in the white population in the United States is 33-39% for men and 23-28% for women.[7]

BCC is a slow growing, locally invasive epithelial tumour that may cause significant tissue destruction and patient morbidity. It is therefore important that BCC is diagnosed early and managed properly.[2,8] Metastasis of BCC occurs only rarely, with reported rates varying from 0.0028% to 0.55%, and has been suggested to be associated with large tumours (>10 cm), tumours with deep penetration, perineural and blood vessel invasion, metatypical tumours, previous radiotherapy and multiple recurrences.[4,9] Recognized patient risk factors are: male gender, red hair, fair skin and blue/green eye colour, higher social class, northern European ancestry and inability to tan easily.[10] The occurrence of consecutive tumours is common. In three years the risk of a patient with BCC to present with another lesion varies from 27 to 44%, reaching 50% in five years. With each new tumour the risk increases further.[11-13]

Aetiology

BCC is understood to result from an interaction between environmental exposures and genetic factors and may be associated with certain genodermatoses.[2,13-15] It is believed to derive from the immature, non-keratinizing cells located in the follicular bulges and the interfollicular basal layer of the epidermis.[2] Cancer is the result of the cumulative effect of various types of damage to the cell's deoxyribonucleic acid (DNA) and signalling systems. This can lead to uninhibited proliferation and growth and loss of regulatory pathways, such as apoptosis (programmed cell death).[16]

Exposure to ultraviolet radiation (UVR), particularly the ultraviolet B spectrum (280-320 nm), is a key risk factor associated with the genesis of BCC.[17] UVR generates mutagenic DNA-products, cytotoxic and mutagenic free radicals and causes immunosuppression of the skin, harming the local antitumour monitoring activity.[13,17-19] The association between UV exposure and development of BCC appears to be complex as the timing, pattern and cumulative exposure to UVR all seem to play a role. Nodular BCCs are more associated with chronic exposure, while superficial BCCs are more associated with intermittent and intense sun exposure. Recreational sun exposure to high doses of UV rays in childhood or youth is a strong risk factor. The greater the number of sunburn episodes experienced before the age of 15, the greater the risk of tumour development.[10,20,21] However, since BCC also may arise on skin not exposed to sunlight, factors other than UVR may contribute to BCC development. Among such factors are exposures to toxic substances (arsenic and coal tar derivates), foci of inflammation (chronic wounds, burn sites), ionizing radiation and conditions that weaken the immune system such as treatment with immunosuppressive drugs.[22,23]

The oncogenesis of BCC may involve various genetic and molecular pathways as well as cellular changes, but is presently considered to depend primarily on the failure of tumour suppression.[4,18] UVR can damage tumour suppressor genes such as the p53 gene, which is involved in surveillance of the regulation of cell proliferation and death. Mutations in the p53 gene are present in more than 50% of BCCs. However, this gene is more closely related to the progression of this tumour than to its origin.[19,24] The tumour suppressor patched (PTCH) gene, originally identified in *Drosophila melanogaster*  (fruit fly), is important for appropriate activation of the hedgehog signalling pathway,

implicated in the formation of embryologic structures and in tumour genesis. Dysregulation of this pathway is thought to be important in the development of BCC. Mutations in the homologue PTCH 1 segment gene located on the q arm of chromosome 9 are associated with BCC in almost all cases of Gorlin-Goltz syndrome (Gorlin syndrome, nevoid BCC syndrome, basal cell nevus syndrome, multiple BCC syndrome), and this association has been vital for the understanding of BCC pathogenesis. Loss-of-function mutations of the PTCH gene also appear in 30 to 70% of sporadic BCC cases.[4,14,18,25] Gorlin-Goltz syndrome is inherited in an autosomal dominant manner and its main clinical manifestations include multiple BCCs, multiple odontogenic keratocysts of the jaws, palmoplantar pits and skeletal abnormalities.[26] Heritable conditions such as Gorlin-Goltz, Bazex, Rombo and xeroderma pigmentosum syndromes predispose to development of BCCs, and should be considered when there are multiple BCCs at a young age.[4,26,27] Genome sequencing studies have also identified mutations in genes involved in pigmentation, DNA repair and immune response, and these mutations are more prevalent in patients with BCC than in controls.[4,19] Higher levels of the apoptosis inhibitory bcl-2 protein have also been found in sporadic BCCs, particular in superficial and nodular types of this tumour.[2]

Clinical presentation

BCC can be classified into a number of different types of which the three main recognised clinical subtypes are: nodular (noduloulcerative, "rodent ulcer"), superficial (multicentric) and morphoeiform/infiltrating (morpheic, mopheaform, sclerosing) **(Figure 2).**[2] The majority of lesions develop on sun exposed skin. About 50 to 80% are located on the face (30% nose) and neck, 11 to 47% on the trunk and extremities and a small number in photoprotected and intertriginous areas.[4,20,28] The frequency of the clinical types differs among populations. The most common form is the nodular type. Both nodular and morphoeiform types predominate on the head. The superficial type is the second most common form and most often appears on the trunk.[4,20]

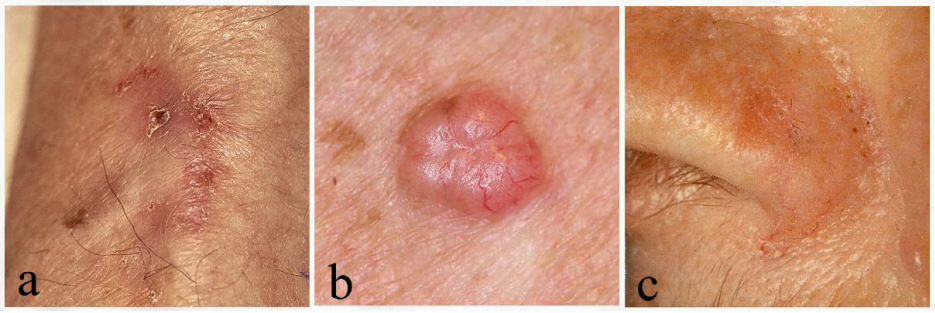


Figure 2.

Clinical presentations of the most common types of BCC: (a) superficial, (b) nodular and (c) morphoeiform. (Provided by Christensen E.)

The superficial type may present as a relatively flat, erythematous plaque, at times with a slightly elevated, translucent threadlike border, and is often multifocal. A few scales may be present and areas of atrophy and hypopigmentation may be seen. The nodular type often appears as an isolated, pearly pink or flesh coloured papule or nodule with surrounding telangiectasias. Lesions may have a translucent or slightly erythematous rolled border and show occasional bleeding, scaling, crusting or ulceration. BCC, mainly superficial and nodular types, may in a few cases be brown-black pigmented in some or all areas. The morphoeiform/infiltrating type most typically presents clinically as an ivory or yellowish irregular shaped, depressed scar with ill-defined borders. Not all BCCs have a clear-cut clinical appearance, as demonstrated by the fact that the clinical diagnosis is not always consistent with the histopathological report.[29,30]

Histological presentation

At present no universally agreed histopathological classification exists for BCC.[31] In 1978 Wade and Ackerman[32] described 26 histopathological variants of this tumour. Other authors have since suggested a simplified classification that is easier to use in both clinical and histopathological practice.[33] Identification of the different histological

subtypes based on tumour growth pattern, can be done on routine haematoxylin, eosin and saffron (HES) stained slides from biopsy specimens. Based on its architectural pattern, BCC can be divided into different histological subtypes **(Figure 3).**

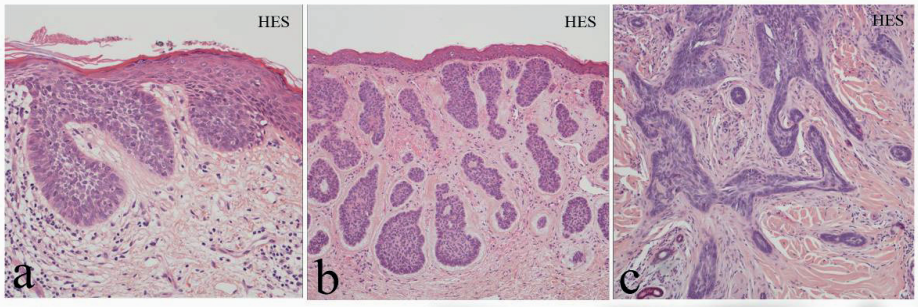


Figure 3.

Histopathological patterns of common subtypes of BCC: (a) superficial (x200), (b) nodular (x100) and (c) morphoeiform (x100). (Provided by Bofin AM.)

The most common subtypes include the superficial, nodular, micronodular, morphoeiform/infiltrating and metatypical types.[2] Histologically, the superficial type shows nests of tumour cells that are in continuity with the epidermis. The nodular type typically presents with large islands of tumour cells with smooth borders where the tumour cells tend to lie in a so-called palisading fashion. The islands of tumour cells are often detached from the tumour stroma (stromal retraction). The morphoeiform/infiltrating type is characterized by the presence of irregular nests and strands of atypical basal cells embedded in a dense fibrous stroma. The extent of infiltration may not be readily appreciated on clinical inspection. It may invade deeply into subcutaneous fat and the risk of subclinical spread is higher with tumours of this type.[34] The micronodular type consists of small nodules and is considered to be more aggressive than the nodular type. The metatypical type is a rare, aggressive type of tumour with characteristics of both BCC and squamous cell carcinoma. In more than 40% of cases BCC presents with a mixed histological growth pattern.[35]

One of the main purposes in classifying BCC is to be able to correlate different subtypes with biological behaviour, thereby providing the clinician with prognostic information useful in the management of these cases.[33,36] Nodular and superficial BCC are commonly regarded as non-aggressive, whereas morphoeiform/infiltrating, micronodular and metatypical types are categorised as aggressive.

Cytological presentation

Skin scrape, imprint and fine needle sampling from BCC may give cellular smears comprising cohesive, anastomosing sheets of small epithelial cells or club-like formations with a high nuclear-to-cytoplasmic ratio and indistinct cell borders **(Figure 4).**

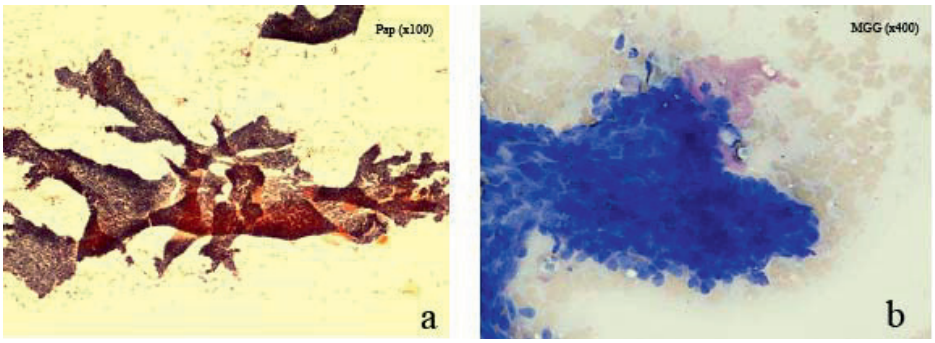


Figure 4.

Cytological presentations of skin scrape in BCC: (a) sheets of atypical basal cells forming club-like structures with smooth peripheral edges and (b) club-shaped fragment of tumour tissue with atypical basal cells with peripheral palisading and an area of pink stroma adjacent to the tumour cells. (Provided by Bofin AM.)

At the edges the cells often appear to be slightly elongated with focal peripheral palisading of the nuclei. There is little variation in nuclear size and shape. However, the nuclei are hyperchromatic, round or oval in shape with finely granular chromatin and

small nucleoli. Nuclear moulding may be seen. Occasional, pink-staining fragments of basement membrane matrix may be observed. Keratinised squamous cells and melanin may also be seen. The basaloid cells of BCC have some similarities with cytological material from skin adnexal tumours such as trichoepithelioma, trichoblastoma, syringoma and metastatic small cell carcinoma.[37,38]

Diagnosis

BCC should be carefully examined before the choice of treatment is made. Clinical diagnosis of BCC is mainly established by means of inspection and palpation of the lesion. The proportion of clinically diagnosed BCCs without histological confirmation differs between countries and has been shown to be 0.7, 7.1, 17.4 and 24.1%, in Malta, the Netherlands, Finland and Scotland, respectively.[39] Agreement between the clinical and histopathological diagnosis of BCC has been found to be from 64 to 98%.[29,30,40,41] It has been shown that variation in the sensitivity for clinical diagnosis of BCC depends on the experience of the clinician with a rate of 89% for skin cancer doctors versus 79% for general practitioners.[42] In a prospective study among plastic surgeons, 70% of lesions suspicious of BCC were confirmed on histology.[43]

Dermoscopy (dermatoscopy, epiluminescence microscopy) may add diagnostic information.[44,45] It is a non-invasive optical magnification tool widely used in the clinical setting. The magnification provided may vary depending on the instrument, and the penetration depth reaches approximately the level of the papillary dermis. A 10-fold magnification is standard for hand held devices. There are two main types of dermoscopes; non-polarised and cross-polarised types. With traditional dermoscopes the lens is placed directly on the lesion of interest after application of immersion oil. Cross-polarised light dermoscopes are small, more flexible and non-touch devices. Multiple lesions can be examined quickly without interface fluid application. With the use of dermoscopes pigmented BCC may more easily be distinguished from other pigmented skin tumours.[46] The most reliable dermoscopic features of BCC are arborizing vessels, grey-brown ovoid nests of cells and brown to grey/blue discrete bulbous structures

forming leaf-like patterns.[47] Dermoscopic sensitivity for BCC diagnosis have been shown to rage from 87 to 96% and specificity from 72 to 92%.[44]

If a conclusive diagnosis cannot be made based on inspection, palpation and dermoscopic examination, histopathological examination of the tumour may be required. Currently, histopathological examination of the tumour specimen is considered to be the "gold standard".[44] The three most common skin biopsy techniques with respect to BCC are: shave, punch and incisional/excisional biopsy.[48] An incisional/excisional biopsy is often elliptical and performed using a scalpel. It can include the whole lesion (excisional) or part of a lesion or part of the affected area and surrounding normal skin (incisional). The biopsy wound is normally sutured and leaves a scar. Incisional/excisional biopsy has the advantages of providing a substantial portion of the lesion with intact histopathological architecture for further investigation in the laboratory. Shave and punch biopsies are widely used for taking minor tissue samples. Both techniques are relatively easy to perform, but normally require local anaesthesia before use. Shave biopsy is well suited for the investigation of superficial lesions while a punch biopsy is preferred of BCCs that are expected to grow deeper into the skin.[49] Biopsy punches are round knives that vary in size and can penetrate into subcutaneous tissue. Even small diameter tissue samples are adequate for histological diagnosis.[50] Small diameter punches heal well without suturing. However, both punch and shave biopsy frequently leave scars.[48]

The sampling procedure for cytological diagnosis is minimally invasive and normally induces no scar formation. It is a method only occasionally reported in the diagnosis of skin cancer despite its widespread use in the investigation of other types of malignancies.[51] Cytological characteristics of BCC are readily identified by microscopic examination of a cytological smear or imprint if sufficient cell material is obtained. It is easy and rapid to perform and requires minimal equipment. No local anaesthesia is needed. It is therefore an attractive diagnostic method to be considered, particularly with minimally invasive treatment options where cosmetic result is of great importance.

The three most frequently used methods for sampling of cell material are: fine needle aspiration, skin scrape and touch imprint.[37] Fine needle aspiration is most often employed for taking of samples from skin nodules and deeper lesions.[52] Skin scrape and

touch imprint are taken from the surface of the lesion after the removal of the keratotic surface and/or crusts. In skin scrape cytology the cellular material is scraped from the surface of the lesion with a small curette and then smeared onto the glass slide. In touch imprint the glass slide is gently pressed against the surface of the lesion and then lifted away. The sampling of cell material is even more rapid using touch imprint than skin scrape, making this procedure attractive in a busy clinical setting.

Studies of skin scrape cytology in BCC have been carried out using various staining techniques. However, no comparison of diagnostic sensitivity between different stains appears to be reported.[53] Among several staining techniques available, the Papanicolaou (Pap) stain has most frequently been used.[53] This stain requires immediate fixation of the smear in alcohol and is particularly useful in the visualization of nuclear chromatin patterns and to differentiate between keratinizing and non-keratinizing cells. It is also well suited to demonstrating the various degrees of squamous epithelial maturation. Nuclei stain blue and cytoplasmic staining vary from blue/green in immature cells to pink and orange in mature, keratinized cells. The May-Grünwald Giemsa (MGG) stain has also been reported in skin scrape cytology of BCC. This stain is performed on air-dried smears, making it even simpler to use. It is especially helpful in showing the non-epithelial stromal elements of the cell material which appears as a pink, amorphous material. This is often quite difficult to see in Pap-stained smears. Modified MGG stains are available for rapid staining.[54]

In addition to the above described methods, the range of other tools for diagnosis of NMSC, including BCC, is increasing.[44] In the past 10-15 years, a number of non-invasive or minimally-invasive technologies have been developed that also may allow the examination of tumours *in vivo*. Besides the possibility of a diagnosis, they may offer the potential to delineate lesion borders and to monitor results after treatment.[44,45,55,56] The applications of such tools are at present principally limited to specialised centres or research facilities. They vary considerably with regard to skin penetration depth, resolution and clinical applicability.

Optical coherence tomography (OCT) provides cross-sectional tomographic images of skin by reflection of infrared light that is measured and demodulated in a digital form. The layers of the skin as well as adnexal structures and blood vessels can be

visualized. OCT may provide information about tumour architecture and morphology, but no cellular details are seen. High frequency ultrasound (HFUS) uses pulsed ultrasound with frequencies between 20-100 MHz to evaluate skin morphology. The intensity of the echo backscattered from tissue is registered. HFUS imaged skin tumours appear echo-poor in comparison to the surrounding tissue. Images are obtained in vertical sections and penetration depth and resolution is related to the frequency. The advantage of this method is deep penetration, but resolution does not permit differentiation of histological subtypes. Confocal microscopy (CM) or confocal laser scanning microscopy is an imaging technique used to increase optical resolution by elimination of out-of-focus information from the object being studied. Conjugated horizontal planes within the tissue are scanned using a low-power laser light source. It produces views at a very high resolution comparable to routine histology sections. The epidermis and superficial dermis are visualized, but depth of penetration is a limiting factor. Reflectance CM is based on the reflectance, scattering and absorption of monochromatic light by endogenous chromophores like melanin, haemoglobin and other cellular structures, whereas fluorescence CM imaging is based on the visualization and interpretation of intra- and intercellular accumulation of endogenous or exogenous fluorophores. Another related technique is multiphoton laser scanning microscopy, an advanced fluorescence microscopy technique, which could potentially be applied for diagnosis of superficial BCC.[57]

BCC may also be detected by the use of optical spectroscopy techniques such as Raman spectroscopy or by use of pulses of electromagnetic radiation in the frequency range of terahertz. In addition, different fluorescence imaging techniques have large potential for visual demarcation of tumour borders.[44] The British Association of Dermatologists considers investigation by computer tomography or magnetic resonance imaging scanning to be indicated in BCCs where bony, orbit or parotid gland involvement is suspected or where tumour may involve nerves.[58] Computer tomography and magnetic resonance studies of SCC and BCC have shown that positive perineural spread is inversely correlated with 5-year survival rate.[59]

Differential diagnosis

Several tumours of different origin may clinically resemble BCC **(Table 1).**

Table 1.

Clinical BCC differential diagnosis

Superficial BCC	Nodular BCC	Pigmented BCC	Morphoeiform BCC
Actinic keratosis	Dermal nevus	Malignant melanoma	Morphea
Eczema	Sebaceous hyperplasia	Angiokeratoma	Lichen sclerosus
Bowen's disease	Squamous cell carcinoma	Haemangioma	Scar
Psoriasis	Dermatofibroma	Blue nevus	
Tinea corporis	Epidermal inclusion cyst	Seborrhoeic keratosis	
	Furuncle		
	Haemangioma		
	Seborrhoeic keratosis		
	Keratoacanthoma		
	Neurofibroma		
	Trichoepthelioma		
	Molluscum contagiosum		

(Provided by Christensen E.)

Actinic keratosis (AK) is considered in Paper I. AK is a very common skin condition occurring among the elderly, appearing singly or more often in groups in large areas (field cancerization) on chronically sun-exposed skin areas. It represents areas of abnormal keratinocyte proliferation and is widely considered to be precancerous as it can progress to SCC in about less than 1 in 1 000 cases per year.[60] AKs can have a diverse clinical appearance from discrete erythematous to scaly thickened, keratotic lesions.[61] AK may to some extent be confused with superficial BCC. In cases of clinical doubt, either a biopsy for histology or a sample for cytology can provide reliable diagnosis.

Treatment modalities

Complete cure is the primary goal of treatment, but the preservation of normal function, good cosmesis and patient preference are also factors that have to be considered in the choice of treatment.[62] There are many different and well accepted treatment modalities available in the management of BCC such as surgical excision, radiation therapy, curettage and electrodesiccation in addition to cryotherapy, all yielding favourable outcomes.[63-66] Surgical excision and radiation therapy appear to be most effective with Mohs micrographic surgery (MMS) showing the lowest failure rates.[3]

In addition, successful uses of different types of lasers in the treatment of BCC have been described.[67-69] Oral vismodegib is a hedgehog signaling pathway targeting agent recently approved for treatment of advanced BCC. It has an encouraging anti-tumour activity with response rates for locally advanced or metastatic BCC shown in a recent open-label study to be 43 and 30%, respectively. The response in patients with metastatic tumours, however, was partial and serious adverse events were reported in 25% of study patients.[70]

The development of more effective, minimally invasive topical therapies with tissue-sparing properties has increased the number of available options for treatment of BCC.[71] Topical photodynamic therapy (PDT) and imiquimod 5% cream are two such therapies having become established treatments for selected BCCs. Topical PDT is described in more detail in subsequent chapters. Imiquimod is a synthetic immune response modifier acting by stimulating innate and cell-mediated immune responses. Favorable tumour clearance rates in superficial BCC were found after topical use of imiquimod cream formulation five times per week for six to twelve weeks. Various degrees of local skin irritation are expected.[72,73]

Topical 5-fluoracil 5% cream and perilesional or intralesional administration with interferon (interferon alfa-2a/interferon alfa-2b) are two treatment options that are more rarely used although they are both found to be effective, largely in the treatment of selected small, superficial BCC.[71,72] Among investigational agents for topical treatment of BCC are tazarotene, cidofovir, solasodine glycoalkaloids and ingenol mebutate.[74-77]

High and low-risk

One of the main concerns with BCC is the possible risk of local recurrence after treatment. With early diagnosis and appropriate therapy, the prognosis is relatively good. However, some patients have lesions resistant to treatment. Based on certain characteristics, BCC may be divided into high or low-risk types.[58,64,78,79] The highest recurrence rates and morbidity are associated with morphoeiform/infiltrative BCC, in addition to large size (about >2 cm, but varying depending on the localization), long duration, mid-face location, recurrent lesions (failures of previous treatment) and perineural invasion. Low-risk tumours are small, well defined, primary, situated at a low-risk location and with a non-aggressive histopathology. MMS and excisional surgery and to some extent radiation therapy are generally used on high-risk s, while less invasive techniques are usually reserved for treatment of low-risk types.[58] Not all patients qualify for surgery, and excision surgery can in certain anatomic areas be challenging and result in cosmetic disfigurement or complications like scar formation and functional impairment. Topical PDT, with beneficial cosmesis, may in such cases be an attractive treatment option.

Topical photodynamic therapy

Research in Norway

Extensive PDT research has been carried out at the Norwegian Radium Hospital, Oslo University Hospital.[80,81] Professor of Biophysics J. Moan recognized the importance of singlet oxygen in PDT in the 1970s. In the late 1990s Q. Peng et al [82] found evidence of porphyrin production after intraperitoneal injection of 5-aminolaevulinic acid (ALA) into tumours in mice. The Norwegian based pharmaceutical company Photocure ASA, was started in1993 to commercialise and carry out further development of photodynamic technologies. Methyl aminolaevulinate (MAL), a lipophilic derivate of ALA, shown to be more selectively taken up in BCC than ALA, was developed.[83] Dr. T. Warloe started clinical studies using topical PDT in the treatment of BCC and showed that supportive tissue penetrator enhancers and curettage improved the outcome, particularly in nodular BCC (1995).[84] Further studies on ALA- and MAL-

PDT in BCC was carried out by dr. A.M. Solér (2002), who demonstrated high complete response rates even after treatment of nodular tumours.[85] From September 1997, the Department of Dermatology, St. Olavs Hospital, Trondheim University Hospital, started treating NMSC with topical PDT on a regular basis. Treatment procedures were carried out in accordance with the then current practice at the Norwegian Radium Hospital, Oslo University Hospital.

Mechanism of action

Topical PDT is based on tissue destruction caused by the interaction of light with a photosensitizer with relatively selective uptake by malignant cells and oxygen to produce reactive oxygen species (ROS), such as cytotoxic singlet oxygen **(Figure 5).**[83] PDT effect is not only confined to the direct, targeted destruction of cells at the treatment site. The release of cell fragments, cytokines and inflammatory mediators that activates the host's innate and adaptive immune system, may lead to further cell destruction.[86]

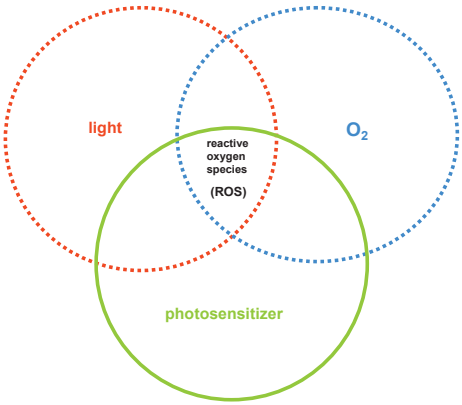


Figure 5.
Mechanism of action of PDT. (Provided by Mørk C and Christensen E.)

Photosensitizers

Photosensitizers are substances that absorb light energy and transform this energy into chemical reactions. Several dyes are applicable as photosensitizers in PDT, but only a few are commonly used in clinical trials and standard PDT treatment of BCC.[87,88] Systemic photosensitisation is avoided in topical-PDT.[83,88] The most frequent, topically applied substances used for treatment of BCC are ALA and MAL. These substances are not photosensitive themselves but enter the haem biosynthetic pathway. Haem is synthesised in mammalian cells. After cellular uptake, MAL is rapidly demethylated to ALA. ALA is a haem precursor, and is metabolized to photosensitive porphyrins, particularly protoporphyrin IX (PpIX). When irradiated and in the presence of oxygen, PpIX is excited and produces ROS, mostly cytotoxic singlet oxygen, leading to cell death by necrosis and apoptosis, depending on the amount of ROS formed **(Figure 5).**[89] Cell necrosis is a pathological process generally associated with severe cellular trauma, which leads to cell death. Apoptosis is programmed cell death characterized by cell membrane changes, nuclear shrinkage and DNA fragmentation. The genotoxic and mutagenic potential of topical PDT is considered to be insignificant. PpIX is mainly formed in the inner mitochondrial membrane. The action radius of singlet oxygen is very short, thus the induced photochemical reaction hardly affects directly on the cell nucleus.[90]

The first of several steps in haem synthesis comprises the condensation of glycine and succinyl-CoA to ALA. Formation of porphobilinogen and subsequent steps in the synthesis lead to the build-up of PpIX. The final step to form haem is the incorporation of iron catalyzed by ferrochelatase into PpIX **(Figure 6).**[83] The haem biosynthetic pathway is normally regulated under close feedback control. Haem inhibits the formation of ALA. When ALA is given in excess, ALA bypasses the cellular feedback control system leading to the accumulation of photosensitive porphyrins in the mitochondria, lysosomes and membranes of the cell. The PpIX synthesis and accumulation is higher in BCC compared to normal tissue. This is due to factors such as increased ALA and MAL penetration through the defect skin barrier in tumours and alteration of cellular enzyme activity in neoplastic tissue. Increased porphobilinogen-

deaminase and decreased ferrochelatase enzyme activities contribute to higher accumulation of PpIX.

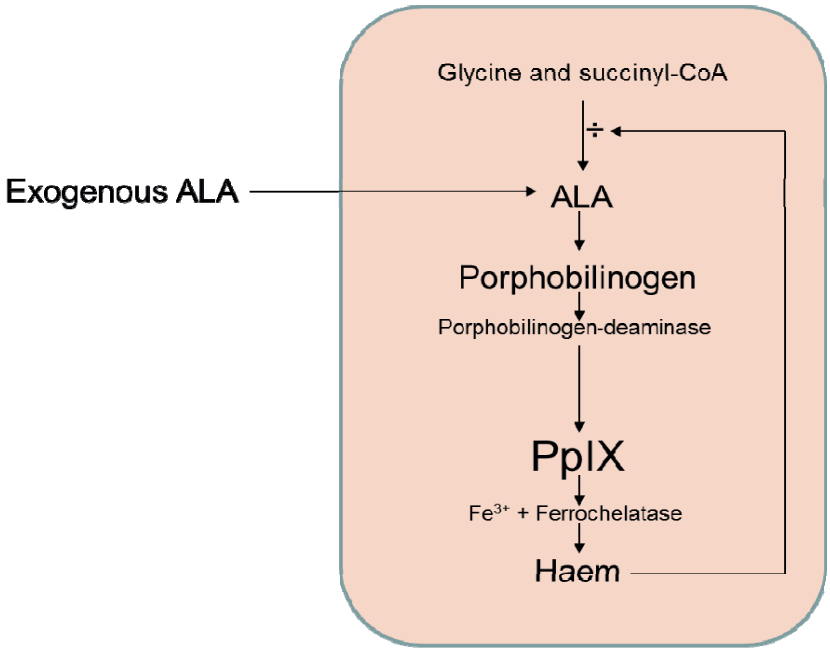


Figure 6.

Simplified presentation of the haem biosynthetic pathway. (Provided by Christensen E.)

ALA is a hydrophilic molecule, which limits its ability to penetrate through cellular membranes. MAL, an ester of ALA, is more lipophilic and may have higher tumour selectivity than ALA.[91,92] However, no difference between transdermal penetration of MAL and ALA has been shown in BCCs [93], and similar effectiveness of MAL- and ALA-PDT has been found after treatment of this type of skin cancer.[94]

Light sources

Broadband and narrow band light sources such as halogen lamps and light emitting diode (LED) lamps, lasers or daylight, may be used for topical PDT. Halogen lamps are cheap and have been widely used, but overheating of the skin may be a problem unless the lamp is equipped with optical filters. The LEDs are simple to use and give off less heat. The exposure time to both halogen lamps and LEDs is usually about 10 minutes.[88] Fractioning of light exposure may improve treatment response by promotion of the photodynamic reaction.[95] Lasers provide the exact selection of wavelengths that match the absorption peaks of the photosensitizer and allow shorter treatment time. Daylight-PDT is currently used for treatment of AK and is also considered in the management of BCC. It is more time-consuming than using traditional light sources, but has the advantage of an almost total elimination of treatment related pain sometimes experienced with the other light sources and may improve the logistical challenges associated with traditional PDT.[96]

The light source used with PDT must emit wavelengths in the absorption spectrum of the photosensitizer. PpIX has a high absorption peak at about 405 nm (blue light) and several smaller bands at approximately between 510 and 630 nm (red light). Most light sources take advantage of the 630 nm absorption peak of PpIX to optimize tissue penetration.[83,88] Long wavelengths penetrate more deeply into tissue than short wavelengths, and the degree of photoactivation is dependent on the amount of light that reaches and is absorbed by the photosentiziser. For thin, epithelial lesions blue light may penetrate sufficiently deep into the skin. In the treatment of thicker BCC, however, a red light source is preferred as it allows a deeper penetration of light into tissue. However, due to light scatter in skin, therapeutic red light extends only to a depth between 1 to 3 mm.[97] The contribution of emission wavelengths beyond 630 nm that may activate porphyrin photoproducts is not known.[98]

Under exposure to blue light the PpIX enriched tumour can be visualized by fluorescence.[99] Fluorescence is the property of a substance to absorb light of a short wavelength and emit light of a longer wavelength. PDT fluorescence can be used in photodetection of malignancies and to delineate poorly defined lesions.[100]

Advantages and disadvantages

Topical PDT is a relatively selective, tissue sparing, non-invasive treatment for BCC. This contributes to a favourable post-treatment cosmetic outcome.[87] It is particularly suited for treatment in patients with multiple co-morbidities, high risk of postsurgical scarring with or without functional impairment, multiple and/or large lesions, in cases where more invasive treatments are contraindicated and for lesions located to sensitive skin areas where cosmetic outcome is of major concern. Several lesions may be treated simultaneously and the same lesion can be treated repeatedly with success if required. Topical PDT may also be used with success in combination with other treatment modalities.[101,102] As topical PDT usually is performed in a medical setting, patient compliance to treatment is high.

Frequent local skin reactions to the treatment site are erythema and oedema. Some patients experience treatment-related pain that usually ends shortly after therapy. The mechanism of this pain is not fully understood. It may be associated with stimulation of nerves through receptors located at the endings of myelinated A delta and unmyelinated C fibres, and with factors such as local hyperthermia and inflammation of the treatment area. There are a number of actions that can control mild to moderate pain such as the use of cold water and/or cooling fans, whereas infiltration anaesthesia or nerve block can be used for management of severe pain.[103,104] Allergic reactions to ALA or MAL are rare.[105] A challenge with topical PDT is the treatment of thicker tumours.[106-108] The method has limited skin-penetrating abilities with ALA and MAL shown to penetrate BCCs efficiently only to a depth of approximately 2 mm [91,93,109], and there is also a limitation of tissue penetration by red light. Topical PDT is not considered to be the treatment of choice for the aggressive and pigmented BCC subtypes.[83,110] Aggressive BCC subtypes tend to show a local invasive behaviour and are surrounded by abundant collagen fibres which may reduce penetration of topical ALA and MAL.[79] In pigmented BCC, optimal light penetration may be inhibited by melanin molecules.[110] PDT performed as mono-therapy does not provide tissue specimens for histopathological examination and confirmation of the clinical diagnosis before treatment.[54,62,105] The standard procedure of topical PDT can be regarded as both time- and resource-consuming. The application time for MAL is routinely 3 hours, which is

the time needed for advantageous porphyrin synthesis and distribution in the tumour before illumination.[111] In addition, topical MAL-PDT for BCC is currently approved for two treatment sessions one week apart.[87,112]

Early clinical experience

Topical PDT has increasingly been used in the treatment of BCC since the pioneer work published by Kennedy in 1990.[113] Early clinical PDT studies demonstrated promising treatment outcome, superior cosmesis and few side effects.[106,113] In 12 ALA-PDT studies carried out from 1990 to 1995 reviewed by Peng et al.[83], the average clearance rate was 87% in superficial BCC, but with lower response rates in nodular tumours. Morphoeic and pigmented lesions responded inadequately to topical PDT. There was no standardised treatment protocol. Most early studies used 20% ALA in an oil-water emulsion, application time varied from 3 to 8 hours and different light sources were used.[83,114] Only one treatment session was usually performed, although some studies suggested improved outcome by repeat treatment sessions.[106,115,116] Histopathological confirmation of BCC clearance after treatment was rarely obtained. In an early study by Calzavara-Pinton et al.[106], treatment areas were surgically removed one month after ALA-PDT. Remnants of BCC, particularly nodular tumours, were found in several of the areas in clinically complete remission. Early studies also demonstrated that clearance rates depend on follow-up time; 75-100% after 1-3 months decreasing to 50-92% after 7-36 months.[83,106,117,118]

Recurrence rates for BCC after traditional treatments have been shown to depend on the length of follow-up, and recurrences have also been shown to occur many years after therapy.[63,64] The slow growth rate of BCC is claimed to be a factor that makes even 5-year follow-up after treatment inadequate.[63] Prolonged clinical and histological follow-up data on topical PDT in BCC is therefore required for evaluation of its long-term effect, and needed for comparison with standard therapeutic procedures.

Tumour thickness

A challenge in the treatment of nodular BCC is to ensure penetration of ALA and MAL into the full thickness of the tumour. The inferior topical PDT response of nodular compared to superficial BCCs led Warloe et al.[119,120] to attempt new strategies to optimize treatment effect, including the use of tissue penetration enhancers such as dimethylsulfoxide (DMSO) and pre-PDT curettage.

DMSO is a chemical solvent with a wide range of physical and chemical properties. It can disrupt the skin barrier through extraction of skin lipids and denaturation of the stratum corneum proteins, thereby permitting increased drug uptake. In addition, DMSO can carry other drugs with it across membranes, and has been shown to increase the permeation of 5-ALA through hairless mouse skin and to initiate haem biosynthesis and endogenous porphyrin production.[121-123] Using DMSO supportive topical ALA-PDT in combination with a porphyrin production inducer (ethylenediaminetetraacetic acid, EDTA), Warloe et al.[119] achieved an improved clinical result for both superficial and nodular BCCs. Tumour clearance rates increased from 67 to 90% for tumours less than 2 mm thick, and from 34 to 50% for thicker BCC.

Curettage is a physical method aimed to remove superficial, hard keratotic tissue and the upper part of the epidermis from the lesion surface before treatment. Thicker BCCs may additionally require intratumoural curettage (debulking), where loose-meshed tissue is removed, reducing the thickness of tumour.[124] Curettage prior to topical PDT may improve ALA and MAL tumour penetration.[105,125] Thissen et al.[126] reported 92% histological clearance in 24 nodular BCCs using PDT with prior debulking. Warloe et al.[120] demonstrated that by performing a simple curettage prior to ALA-based topical PDT the complete response rate for thick (> 2 mm) BCCs increased from about 50 to 89%.

According to the international consensus guideline, PDT is recommended as a treatment option for superficial and thin nodular BCCs (thickness < 2 mm).[87,112] Some investigators, however, accept 2-3 mm thick BCCs for PDT when combined with prior curettage (debulking).[87,110] To achieve a good result it is important to identify those tumours most likely to respond to treatment. Careful selection of BCC, with thickness up to about 2 mm, for treatment may thus increase the success rate of topical PDT in

BCC. Although BCC characteristics in daily practice may be assessed by clinical examination, the histological examination of biopsy specimens is perceived to give a more accurate measurement of tumour thickness.[62] Such information can prove important in the evaluation and selection of BCCs most suited for PDT. The taking of a pre-treatment biopsy is therefore encouraged.[62,105,127] A limitation of punch biopsy, however, is that it will only offer information from a small, selected area compared to an excision specimen which allows a more extensive examination of the tumour.

AIMS OF THESIS

The general aim of this thesis was to study selected diagnostic methods for BCC in relation to topical PDT and long-term treatment results. This was done by investigating various aspects of cytological diagnosis in BCC and whether punch biopsy provides sufficient information about BCC tumour thickness for adequate selection of lesions suitable for treatment with topical PDT. The long-term treatment results were obtained by regular and careful prospective clinical and histological follow-up of the treatment areas in patients having received curettage and topical PDT for BCC.

Specifically, we sought to:

- Evaluate the diagnostic performance of skin scrape and touch imprint cytology in BCC.
- Compare two different cytological staining methods in the diagnosis of BCC.
- Investigate the agreement between tumour thickness measurements in paired punch biopsy and excision specimens from individual BCCs.
- Investigate 6-year treatment efficacy and cosmetic outcome in treated areas clinically considered to be in complete remission at 3 months after curettage and one or two sessions of topical DMSO-ALA-PDT in BCC.
- Describe BCC tumour recurrence over time after treatment by curettage and topical DMSO-ALA-PDT, and explore clinical and histopathological factors associated with treatment failure.
- Investigate 10-year treatment efficacy and cosmetic outcome of primary and recurrent BCC after curettage and one or two sessions of topical DMSO-ALA-PDT.

MATERIAL AND METHODS

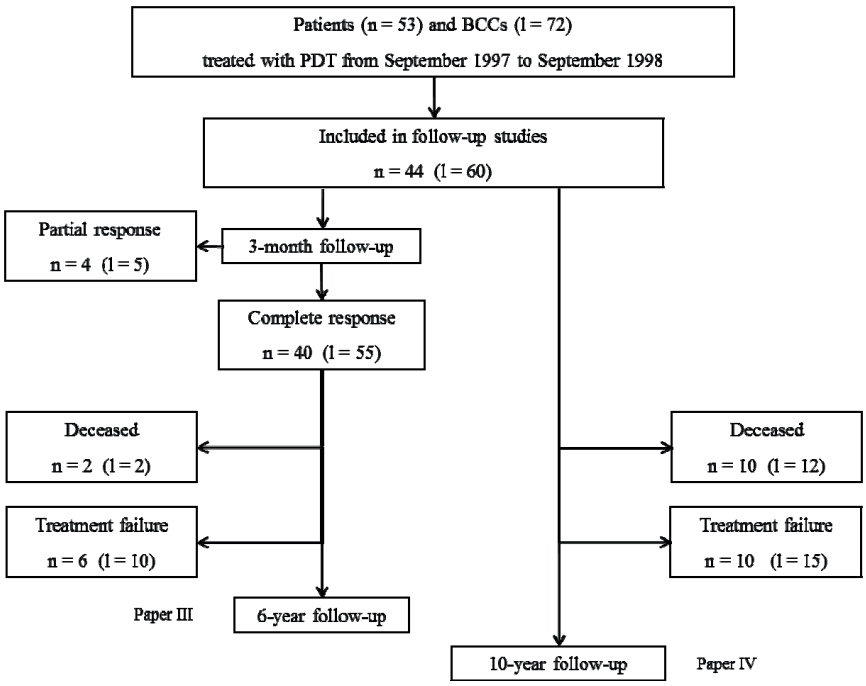
All patients were referred to the outpatient clinic at the Department of Dermatology, St. Olavs Hospital, Trondheim University Hospital. Different cohorts of patients were studied in paper I, II and III/IV. BCC and AK lesions were histopathologically confirmed. In Papers I, III and IV the skin lesions were treated with topical PDT. In Paper II, the selected method of treatment was surgery. Specific inclusion and exclusion criteria were defined (See enclosed publications for details).

In Paper I, we compared cytodiagnostic skin scrape and touch imprint results with the histological diagnosis in 50 BCC and 28 AK lesions from 41 and 25 patients, respectively. All cytological specimens were examined in a random and blinded fashion by two pathologists. The histopathological diagnosis was considered to be the "gold standard".

In Paper II, we compared tumour thickness measurements of individual tumours in punch biopsy and the corresponding excision specimens from 48 BCCs in 43 patients.

In Paper III, we performed a long-term (6 years) follow-up of 60 BCCs in 44 patients treated with one or two sessions of topical DMSO-ALA-PDT following curettage which clinically appeared to be in complete remission 3 months after therapy **(Figure 7).** At the beginning of the study, in September 1997, all lesions received two treatment sessions one to two weeks apart. From February/March 1998, only one session was given. Evaluation of the treated areas was done at regular intervals; 3, 6, 12, 24, and 72 months after treatment. The post-treatment follow-up period was initially intended to be one year, and was thereafter gradually prolonged.

In Paper IV, we followed the same cohort as described in Paper III, up to 10 years after treatment **(Figure 7).**



n = number of patients

l = number of lesions

Figure 7.

Flow chart with number of patients and lesions in Paper III and Paper IV. (Provided by Christensen E.)

Procedures

In paper I, a curette was used to gently scrape the surface of a lesion. Then the cell material was deposited on a glass slide and spread directly with a second slide. From an individual lesion, the first skin scrape smear was stained with Pap and the second was stained with MGG. A microscope slide was then firmly pressed against the lesion surface to make an imprint. The imprints were stained with MGG **(Figure 8).**

Disposable biopsy punches of 2, 3 or 4 mm in diameter were used to obtained tissue specimens for histopathological investigation (Papers I-IV). Biopsy tissue was routinely fixed in 4% formaldehyde and further processed before embedding in paraffin wax. Sections were cut at 4µm and stained with HES and examined under a microscope.

In Paper II, a punch biopsy and an elliptical surgical excision were used to provide tissue for histopathological investigation. After fixation in 4% formaldehyde the excision biopsies were cut into several slices before processing following the same procedure as for punch biopsies.

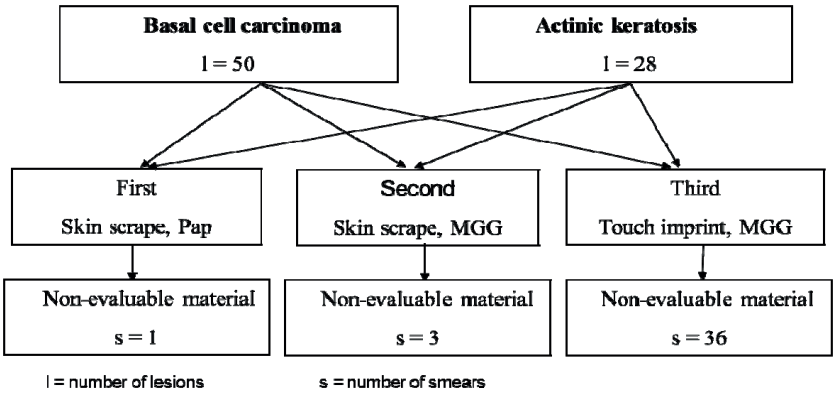


Figure 8.
Sampling and staining methods for cytological investigation of BCC and AK and numbers of non-evaluable smears. (Provided by Christensen E.)

The punch biopsy for investigation of tumour thickness was taken from the part of the tumour clinically considered to be thickest. If the tumour on clinical inspection and palpation appeared of homogenous thickness the biopsy was taken from the central area. After obtaining the punch biopsy, an elliptical excision of the whole tumour was performed **(Figure 9).**

In Papers III and IV, curettage of BCC and the surrounding skin was performed on all lesions. Additional careful intratumoral curettage (debulking) was included for those cases in need of tumour volume reduction.

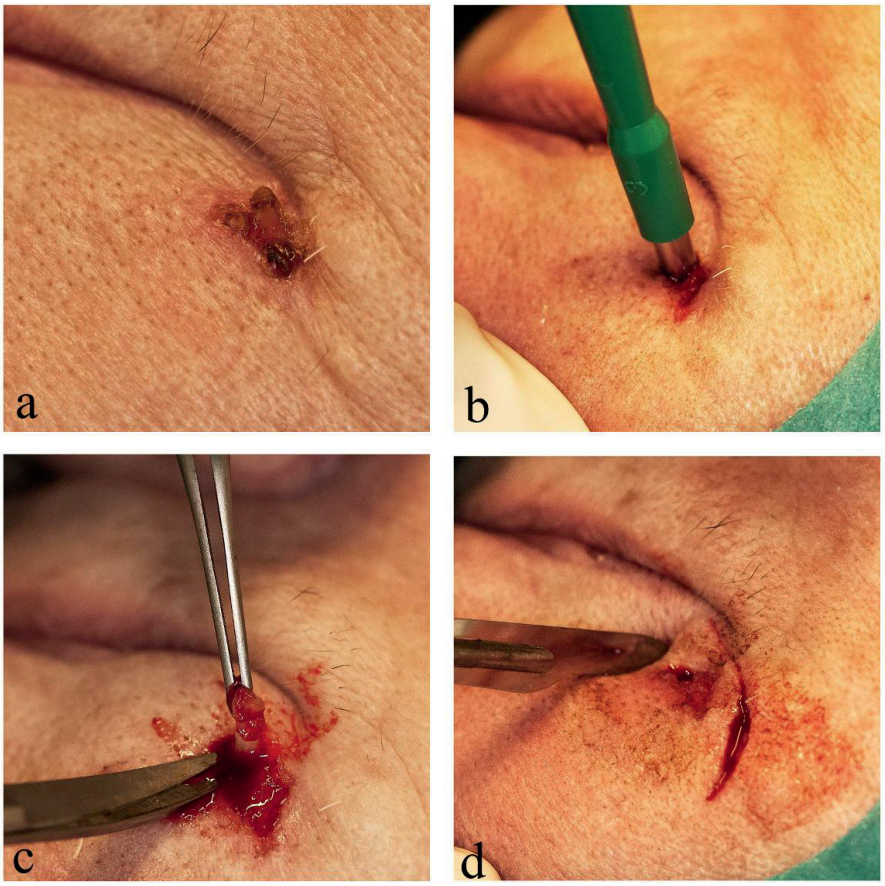


Figure 9.

The sequence of paired punch and excisional biopsies: (a) noduloulcerative BCC, (b) 3 mm punch biopsy, (c) punch biopsy specimen and (d) elliptical excision. (Provided by Christensen E.)

Drug formulations and light source

Three types of drug formulations were used. In Papers III and IV, gauze soaked in DMSO 99% was applied on lesions located on trunk and extremities for 5 minutes before use of ALA. An emulsion of ALA 20% in Unguentum Merck was then applied

for approximately 3 hours before further treatment with light. On lesions located on the face and hairy scalp, DMSO 4% was added to the ALA-containing emulsion. A broadband lamp developed at the Norwegian Radium Hospital with an emission spectrum of 550-700 nm and light intensity at the skin surface of about 150 to 230 mW/cm2 was used.

Lesion evaluation

Histological and cytological evaluations of BCC and AK were made in Paper I. In Papers II-IV, clinical and histological evaluations of BCC were used. Clinical evaluation of BCC and of treatment sites was performed through inspection and palpation (Papers II-IV). Lesion size was clinically estimated as the mean value of the greatest length and width. The H-zone was defined as the area of the face and ears corresponding to the area marked grey on a figure sketch (Paper III) **(Figure 10).**

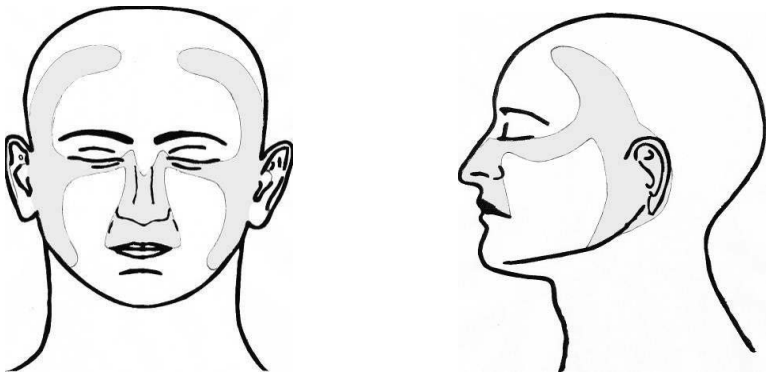


Figure 10.

Anatomical depiction of the "H-zone" marked as a grey area. (Provided by Christensen E.)

Cosmetic outcome was assessed at the follow-up visits by the investigator and recorded on a 4-point scale as excellent, good, fair or poor (Papers III and IV). The

patients evaluated the cosmetic outcome at the 6-, 12-, 24- and 36-month visits using the same assessment scale.

A punch biopsy for histopathological examination was taken at follow-up from all treatment areas clinically suspicious of treatment failure. In addition, treated areas clinically considered to be in complete response were biopsied for histological investigation at the 12- and 36-month follow-ups (Papers III and IV). Based on histopathological investigations of biopsy specimens, BCCs were subtyped by different classifications methods. In papers I, II and IV, the classifications were based on histopathological growth pattern of tumours.[33,128] In Paper III, BCC tumours were subclassified as either superficial or nodular type according to whether growth extended to or deeper than the papillary dermis.[129] Tumour thickness was measured from below the stratum corneum to the base of the deepest tumour nest to the nearest 0.1 mm (Paper II).

The cytological features of BCC were based on the presence of tight groups of atypical, uniform, small cells, as described in more detail in the introduction part of the thesis. The features of AK were based on findings of individual and groups of dysplastic keratinocytes often with ragged edges. Intercellular bridges could be seen. The cells showed a polyhedral or spindle-shaped configuration and the nuclear-to-cytoplasmic ratio was moderate to high. The results of the cytological evaluations were grouped into four categories: BCC, AK, non-BCC/non-AK and non-evaluable.

Statistical methods

Continuous variables were reported as mean, standard deviation and range. Categorical variables were presented as numbers and/or percentages. Normal distribution was assumed when using parametric tests. In Paper II, a paired-sample t-test was used to compare the mean of two variables. Agreement between punch biopsy and excision specimen tumour thickness measurements was analysed using a difference-versus-mean ("Bland-Altman") plot.[130] The 95% prediction limits for excision tumour thickness given punch biopsy measurements were obtained by use of a regression approach that takes an increasing standard deviation into account.[131] In this study a one-sided

prediction limit was considered most informative with respect to clinical interpretation. Chi-square test of association was used in the analyses of association between treatment complete response and treatment failure (categorical variables) (Paper IV) and McNemar's test was used for testing an association between matched pairs (Paper 1). The cytodiagnostic performance was expressed as point estimates of sensitivity and specificity with 95% confidence intervals using the binominal distribution (Paper I). Estimation of probability of treatment failure was conducted using time to event (survival) analyses that account for censored observations and Kaplan-Meier plots (Paper III and IV). Univariate analyses of different factors were done using the log rank test. A Cox model was used to explore the relationship between BCC recurrence and possible explanatory variables. This included patient gender, age, immunological status and lesion site, size, thickness, subtype, primary or recurrent, in addition to treatment related factors as number of treatment sessions and light dose. In Paper IV, the analysis was adjusted for clustering of lesion among patients.

The statistical software R, version 2.5.0 and 2.11.1 and the software SPSS version 15 were used for statistical analyses. P value less than 0.05 was considered statistically significant.

Supplementary statistical analyses

In Paper III, analyses investigating the relationship between BCC recurrence after treatment and possible explanatory variables were repeated using a robust variance estimator that takes clustering of lesions among patients into account. (The outcome is presented under section: Results).

ETHICS

The regional committee for medical research ethics approved all the studies.

The cytological investigation was considered by the committee to be a quality assurance study (Paper I). The cell material provided for cytology was obtained as part of the pre-treatment PDT procedure.

The extension of the follow-up period after topical PDT of BCC may have been a burden on patients. However, prolonged follow-up after treatment of this type of skin cancer can be advantageous as patients with BCC are at higher risk of developing new lesions within a few years. At follow-up, new lesions may be diagnosed and treated at an earlier stage than otherwise would have been the case (Papers III and IV).

Patients who at any time during follow-up were excluded from further study, due to clinical and/or histological BCC recurrence or who wished to withdraw from the study, were offered additional treatment options at the discretion of the investigator (Papers III and IV).

RESULTS

Synopsis of Paper I

Objective:

To evaluate the diagnostic performance of skin scrape and touch imprint cytology in BCC and to compare diagnostic results using two different staining techniques.

Material and methods:

Two skin scrape and one touch imprint were taken from each lesion in 50 BCC and 28 AK lesions. Scrape smears were stained with Pap or MGG stain and imprints were stained with MGG. Cytodiagnostic results were compared to the histopathological report which was considered the "gold standard".

Results:

- The sensitivity and specificity for skin scrape cytodiagnosis using Pap stain in BCC were both 96%.
- The sensitivity and specificity for skin scrape cytodiagnosis using MGG stain in BCC were 94% and 96%, respectively.
- Skin scrape diagnosis differentiated BCC well from AK.
- Touch imprint sampling procedure returned non-evaluable material in 32% and cytological diagnosis agreed with histopathology in 62% (31 of 50) of samples from BCC.

Conclusion:

Skin scrape cytology with either Pap or MGG stain, is a useful method and performs well for the diagnosis of BCC. Touch imprint cytology was not found useful as a routine diagnostic method.

Synopsis of Paper II

Objective:

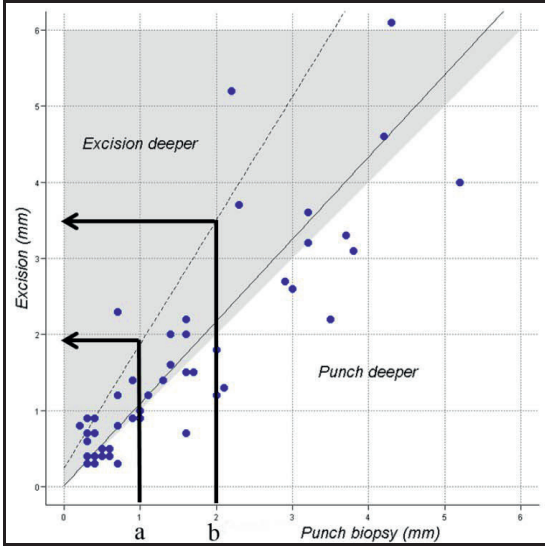
To evaluate information from punch biopsies of BCC with regard to tumour thickness.

Material and methods:

From individual tumours, thickness measurements in punch biopsy and the corresponding excision specimens of 48 BCCs from 43 patients were investigated.

Results:

- Tumour thickness was measured to be 0.14 mm greater in excision specimens than punch biopsies when comparing the mean (average) measurement of the two groups.
- From individual BCCs, corresponding thickness measurements by the two methods showed increasing discrepancy between measurements with increasing tumour thickness **(Figure 11).**



The line formed by the transition between shaded and white area is the line of equality. The unbroken line shows the mean difference between measurements by the two methods. The broken line is the upper 95% prediction line.

Figure 11.
Illustration of the 95% upper prediction limits for excision tumour thickness given the punch biopsy thickness measurements by use of a regression model. Two examples are shown: a) in tumours with punch biopsy thickness of 1 mm, the excision specimen thickness would with 95% probability be less than 2 mm and b) with punch biopsy thickness of 2 mm, the excision specimen thickness would be less than 3.5 mm. (Provided by Skogvoll E and Christensen E.)

Conclusion:
The prediction of "true" tumour thickness from a single punch biopsy of individual BCCs may be uncertain, and prove insufficient for deciding whether or not the tumour falls within the current recommended limit, of approximately 2 mm thickness, for topical PDT.

Synopsis of Paper III

Objective:
To evaluate clinical, histopathological and cosmetic treatment outcome in BCC at regular intervals, up to 6 years after curettage and topical PDT, and to evaluate associations between treatment response and patient and lesion characteristics.
Material and methods:
A total of 44 patients with together 60 histologically verified BCCs were studied after treatment with curettage and one or two sessions of DMSO supportive topical ALA-PDT. Treatment areas in clinical complete response 3 months after treatment were followed regularly for 6 years with clinical inspection, histological investigation of biopsy samples and cosmetic evaluation. Data from deceased patients were not included in the long-term results. Histologically verified BCC recurrences were consecutively excluded from the study.
Results:

- At 3 months, complete treatment response was observed in 92% (55 of 60) of the treatment areas.
- At 72 months, complete treatment response was observed in 81% (43 of 53) of the treatment areas; 68% after one treatment session and 91% after two sessions.
- Recurrence was noted in 2, 4, 2 and 2 tumours at 6, 12, 24 and 36 months follow-up, respectively.
- Clinical assessment of treatment areas identified 9 of 10 BCC recurrences.
- The investigator assessed cosmetic outcome was rated as excellent or good in 91, 97, 97 and 100% at the 12-, 24-, 36- and 72-month visit, respectively.
- The patient assessed cosmetic outcome was rated as excellent or good in 100% at the 24-, 36-and 72-month visits.

- Male gender and H-zone location were each associated with recurrence of tumour.

Conclusion:

Topical curettage and DMSO-ALA-PDT has a favourable long-term outcome in the treatment of BCC. Clinical assessment of the treatment areas appears to be sufficient in long-term follow-up.

Synopsis of Paper IV

Objective:

To evaluate 10-year efficacy of curettage and topical PDT in primary and recurrent BCC, and to evaluate clinical and histological tumour characteristics that may be associated with treatment failure.

Material and methods:

The same cohort as described in Paper III, but also including those patients with lesions showing 3-month partial response to treatment, were followed up to 10-years after curettage and one or two sessions of DMSO-ALA-PDT. Results were reported as sustained lesion complete response rate using a time to event analysis, histologically confirmed treatment failure and assessment of cosmetic outcome.

Results:

- At 10 years, the overall lesion complete response rate was 75%; 60% after one treatment session and 87% after two sessions.
- Lesion complete response rate for primary BCC was 78%; 63% after one treatment session and 90% after two sessions.
- Overall treatment failure was found in 25% (15 of 60) of treated BCC, and all were identified within 3 years of treatment.
- At 10 years, the cosmetic outcome was rated by the investigator as good or excellent in 100% of cases.
- Male gender, recurrent BCC and one treatment session were each associated with treatment failure.
- The only lesion larger than 2 cm in the study relapsed.

Conclusion:

Two sessions of topical DMSO-ALA-PDT with curettage provide high and sustained efficacy with favourable cosmetic outcome in the treatment of small, primary BCC. All treatment failures appeared within three years after treatment.

Supplementary results

By use of a statistical model that takes clustering of lesions into account, male gender ($p = 0.008$) and H-zone ($p = 0.028$) were found to be associated with tumour recurrence, thus leaving the originally reported results unchanged (Paper III).

DISCUSSION

Methods

Paper I reports results from a study comparing different cytological diagnostic methods using histology as the reference. In Paper II, the agreement between two methods of assessing tumour thickness measurement is investigated. In Papers III and IV, the long-term outcomes after use of curettage and topical PDT for BCC are reported in a prospective cohort study.

Dermatologists at the out-patient clinic selected lesions (patients) for the various treatment modalities. This may have resulted in bias, as treatment was not randomly assigned. Thus some patients and certain tumours were less likely to be represented in the studies. For example, the overall distribution of BCC shows a predominance of the nodular type, which most often appears on the head.[20] In Paper II, most of the BCCs were of the superficial type and located to the trunk. We suspect 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 St. Olavs Hospital, Trondheim University Hospital. This may have led to a de-selection of nodular BCCs for PDT, which further may have affected study results, because nodular tumours are presumably thicker than superficial BCCs. If a greater number of nodular tumours had been included, the disparity between the two methods of measuring BCC thickness might have been greater than shown in the study.

The sample size in Papers III and IV was limited to the number of patients who received topical PDT within one year and met study inclusion criteria while avoiding those of exclusion. Fifty-three patients with a total of 72 lesions were treated with curettage and topical PDT at the out-patient clinic from September 1997 to September 1998. A number of these patients and/or lesions were excluded from study follow-up due to various protocol violations; one had a lesion surgically removed, but received PDT most likely to prevent recurrence; one had a pigmented BCC with a thickness of more than 4 mm; one had a tumour that on histology showed a trichoepithelioma; one had a BCC diagnosed from a large leg ulcer which required many consecutive PDT

sessions and one patient was not suited for undergoing the prolonged treatment procedure as required. The remaining were excluded from follow-up because the lesions were not biopsied before treatment, thus no histologically verified diagnosis was available. Even so, the limited exclusion criteria in Papers III and IV allowed a diversity of patients and BCCs to be represented in the cohort, including recurrent lesions and lesions in immunocompromised patients that other studies may have excluded. However, the numbers of patients and lesions in the cohort is relatively small. This may cause low statistical power and in particular limits the potential for subgroup analyses.

The main outcomes in Paper IV were given as point estimates with 95% confidence intervals (CI). A CI is a calculated interval in which the "true" value is contained with a defined degree of confidence, normally 95%. It thus quantifies the uncertainty of an estimate.[132] If the study is repeated many times in the same population, these Cls would contain the underlying "true" value in 95% of the time (given 95% confidence). The width of a CI is a function of the standard deviation of the sampling distribution (standard error) and of the chosen level of significance. The sample size affects the standard error. Therefore, by including more BCC lesions, the width of the 95% CI around the point estimates of lesion complete response rate could have been narrowed, hence, increased the precision of these estimates.

In Paper I, the CIs of the point estimates for BCC skin scrape cytodiagnostic results by both Pap and MGG staining techniques were fairly narrow, indicating that the sample size was statistically adequate. The diagnostic ability of cytology was compared to histopathology, which was considered the "gold standard", and reported as the sensitivity and specificity. A perfect diagnostic test gives 100% sensitivity and 100% specificity. In Paper I, the sensitivity represents the percentage of histologically verified BCCs that were correctly identified by cytology. The specificity identifies the percentage of negatives that were correctly identified as non-BCC.[133] By including histologically verified non-BCCs (AKs), the specificity of cytodiagnosis of BCC could be assessed.

The outcome measures in Paper III and Paper IV were reported somewhat differently. In Paper III, the calculation of 6-year complete response rate did not include data from the five lesions showing partial response to treatment at 3-month follow-up.

This way of reporting treatment results has been commonly used in many PDT studies, but may have led to over-optimistic results.[87,112] Some researchers consider that BCCs reappearing within a treatment field represent a residual lesion rather than a new primary lesion.[63,108,134] In line with this view, both incomplete responders by 3 months following treatment as well as later recurrences were classified as treatment failures in Paper IV. The proportion of treatment failures may appear to be higher by this approach than in similar studies that do not include the early incomplete responders.

Long-term follow-up studies are susceptible to attrition, thus the handling of data from participant dropouts is important in the interpretation of results.
The best method for estimation of lesion response to treatment over time is claimed to be the use of a time to event approach. Such a method has the advantage of being able to use all available data, including data from dropouts, to estimate the complete response probability.[64,66,135] This approach was used in the 10-year follow-up study to estimate treatment response.

A common way of reporting recurrence rates is based on the total number of patients with recurrent BCC divided by the total number of patients with initial lesions treated (raw recurrence rates).[64] This approach ignores the patients lost to follow-up and may give an underestimation of the recurrence rate. Other long-term studies report recurrence rates based on the total number of patients with recurrent BCC divided by the total number of patients with lesions that were evaluated at the final follow-up (strict recurrence rate). This method may artificially raise the recurrence rate because it excludes patients who are cured and whose control period is shorter than the final study control. The actual recurrence rate is believed to be somewhere between the values derived from using raw or strict recurrence rates.

In Paper III, we avoided the uncertainty of treatment response with regard to participant dropouts in the interpretation of our results. Two patients died very early in the follow-up period. No further patients were lost to follow-up. By excluding these two patients in the analyses, all information with regard to BCC recurrence during follow-up was available after the final visit.

According to acknowledged principles of clinical study design, the demonstration of efficacy of a treatment is preferably based on comparing the response in the treated group with that of a control group receiving placebo or another active treatment. Patients should then be allocated at random (randomized) to one of different treatments. Randomized, controlled studies provide the best evidence with minimal bias (systematic skrewness), particularly if blinded.[132] Without a control group, as in both Papers III and IV, both bias and confounding (i.e. error in assessment of cause-and effect) may have affected the results. Even so, these studies may give information on extent and duration of treatment effect and suitable treatment strategies.

Many of the early studies on topical PDT of BCC have a limited follow-up period. The strength of Papers III and IV is the close and long follow-up after treatment. Treatment efficacy was found to be much higher in lesions receiving two treatment sessions compared to those receiving only one. For practical reasons the number of treatments was changed from two to one PDT session halfway through the treatment period of one year. In a sense the allocation to treatment could be regarded as "random" in the popular meaning of the word, as the number of treatment sessions given was not determined by individual considerations, but by the point in time at which the treatment took place. However, since the lesions were not appropriately randomized to one or two treatment sessions the results should be interpreted with caution.

In Paper 1, all the cytological smears were evaluated in a blinded and random order before being compared with the histopathology report that was regarded as the "gold standard". In the interpretation of the cytological smears observer variability may be a source of uncertainty in the evaluation of cytology as a diagnostic test. Thus the question of reproducibility of the results may be raised. Strength of this study is that two pathologists with extensive experience in general cytology evaluated the smears and imprints. Bias may have been introduced when obtaining the cell material, however, as the sequence of sampling may have influenced the quality of the smears and imprints. The first skin scrape smear from each lesion was stained with Pap and the second with MGG. Imprints were always made after skin scrape sampling, which further may have reduced the availability of representative material. This is an evident weakness of this study design.

Results

In Paper I, skin scrape cytological diagnosis showed a high sensitivity and specificity for BCC and differentiated well between BCC and AK. The satisfactory agreement shown between cytological and histological diagnosis in BCC is consistent with the results reported in a systematic review of the literature on this area from 2004.[53] The pooled sensitivity and specificity of cytodiagnosis in BCC was reported to be 97% (95% Cl 94-99) and 86% (95% Cl 80-91), respectively. The studies eligible for this review, including information of the staining techniques that were used in each study, are shown in **Table 2.** The review does not conclude with regard to which of the staining techniques was the most suitable.

Table 2.

Studies of skin scrape cytological diagnosis of BCC where histopathology was used as "gold standard"

Study	Year	Sample size	Staining technique
Brown CL et al.[139]	1979	131	MGG
Gordon LA and Orell SR[137]	1984	150	Pap
Böcking A et al.[144]	1987	37	Pap
Ruocco V[143]	1992	498	MGG/Pap/Giemsa
Derrick EK et al.[142]	1994	246	MGG
Barton K et al.[145]	1996	20	Pap
Berner A et al.[54]	1999	112	Diff-Quick *
Vega-Memije E et al.[136]	2000	15	Pap

*Diff-Quick is a MGG stain variant utilized on material which is air dried (Provided by Christensen E.)

The results in Paper I show that Pap and MGG stain may be equally well used in the diagnosis of BCC. The sampling of cell material on to the glass slides was done after

careful removal of any keratotic layer covering the lesion using a small curette. Only 2.6% of the skin scrapes were considered to be non-evaluable as compared to 4.5, 6 and 13% in previous studies.[54,136,137] Adequate sampling is required to achieve good results as unsatisfactory and non-representative smears can give false negative cytological diagnostic results.[138,139] Slightly more of the MGG stained smears were regarded as non-evaluable compared to Pap stained smears (Paper I) **(Figure 8).** To compare the diagnostic ability of Pap and MGG stain more appropriately, the order of sampling of cell material from individual lesions for Pap or MGG staining should have been randomized. The diagnostic result of touch imprint cytology may, to an even greater extent than MGG skin scrape, have been affected by the order of sampling. Almost one third of the imprints had too sparse material for diagnosis. Of the remaining representative slides, however, 97% of BCCs were correctly identified. Touch imprint cytology has been demonstrated to agree 100% with cytohistopathological examination when smears were prepared by touching the cut surfaces of excised BCCs on to the slides.[140] However in another study, touch imprint cytology was reported to be positive for BCCs in only about 50% of the investigated cases.[141] Further studies are needed to determine the role of touch imprint cytological diagnosis in BCC.

Punch biopsy specimens for histological diagnosis were regarded as "gold standard" for the diagnosis of BCC, and used for cytodiagnostic comparison. This was done even though a small biopsy specimen taken from a selected lesion area may not be representative of the whole BCC. An advantage of skin scrape cytology is that representative material from the entire surface of the lesion may be sampled and investigated. This makes skin scrape cytology particularly useful in diagnosing superficial BCC. If cytology is negative in a lesion clinically suspicious of malignancy, it is still possible to take a biopsy specimen for histological examination. Furthermore, cytological smears can easily be prepared from the curetted and/or debulked material obtained during PDT preparation, to confirm the BCC diagnosis in those cases no prior confirmation of the clinical diagnosis exists. However, skin scrape cytology can not be used to determine BCC thickness and has not been shown to differentiate between BCC subtypes. Cytology also has limitations in differentiating between BCC and other adnexal tumours.[38]

In Paper II, we found an overall reasonable agreement between the mean (average) measurements of BCC thickness of the two groups. This suggests that the mean (average) punch biopsy thickness may be used to approximate the mean (average) thickness of BCC, which can be useful in relation to scientific work. In daily practice, however, the thickness of the individual BCC is of the utmost clinical relevance. BCC thickness has received particular attention along with the introduction of topical PDT. A pre-treatment lesion biopsy for histopathological investigation is recommended by the Norwegian practical PDT guidelines.[105] It is currently a supportive diagnostic method often used in clinical practice and can provide information for selection of BCCs suited for PDT. Based on a systematic literature review, the current international PDT consensus guideline recommends the use of topical PDT for BCCs with a thickness up to 2 mm.[112]

We found that thickness measurements in punch and excision biopsy specimens from individual BCCs may differ considerably and, moreover, that this disparity increased with increasing tumour thickness. Based on the data in Paper II, a pre-treatment tumour thickness biopsy measurement of up to 1 mm suggests that the BCC most likely will be within the limit of the international consensus guideline with regard to thickness. BCCs that on biopsy measure more than 1 mm, however, may well exceed this limit. Thus, information on tumour thickness returned from histological investigation of a biopsy specimen appears, in some cases, to be insufficient for appropriate selection of treatment.

Although not directly comparable to results in Paper II, the relationship between punch biopsy and excision specimen tumour thickness in SCC of the lower lip has been investigated. This study also demonstrated considerable disparity between the two sampling methods. This discrepancy was particularly evident in tumours that were greater than 3 mm thick.[146]

New imaging techniques for non-invasive investigation of skin tumours, such as OCT and HFUS, can be used to estimate BCC thickness.[55,147] In a study comparing HFUS and OCT to determine BCC thickness in 10 lesions, OCT agreed better with histopathological tumour thickness (the "gold standard") than HFUS.[148] Similarly, in another study investigating tumour thickness in 62 BCCs, OCT appeared to correlate

better with histology than HFUS.[55] With HFUS, tumour thickness has been shown to be overestimated, because subtumoural inflammatory infiltrates cannot be differentiated sufficiently well from tumour tissue by use of this technique. OCT, on the other hand has been demonstrated to both over- and underestimate tumour thickness. Due to scatter of light in tissue, the imaging effect of OCT is limited to a depth of approximately 1 mm.[55,149] Hence, OCT is currently insufficient for pre-treatment characterization of BCC thickness. However, it offers the possibility to provide an impression of several morphological characteristics, whereas HFUS does not differentiate properly between different skin lesions. So far, these technologies are considered experimental in the characterisation of BCC thickness. Histopathological examination of BCC is therefore the reference standard.

Punch biopsy is a well established technique used to obtain full-thickness diagnostic tissue.[49] With this technique tissue architecture is well preserved, and even punches with small diameter provide material of sufficient size and quality for reliable histological diagnosis.[50] In Paper II, both punch- and excisional biopsy provided adequate tissue samples for the investigation of deep tumour margin in almost all cases. Nevertheless, the possibility of not establishing the "true" maximum tumour thickness in a given BCC either by examination of punch biopsy or surgical excision specimens remains. A punch biopsy specimen only represents a small part of the total tumour area and, even in excisional biopsy only limited sections of the specimen are prepared for microscopic examination.[150] As the excision in this study always followed immediately after the biopsy punch, any error in the evaluation of tumour thickness due to changes of tumour over time (e.g. regression or growth) was eliminated.

The overall BCC complete response rates after 6 and 10 years were 81 and 75%, respectively (Papers III and IV). All treatment failures were noted within 3 years of treatment. These results are similar to long-term results of other ALA- and MAL-PDT studies and give further evidence in support of topical PDT as an attractive treatment option with sustained high efficacy for treatment of BCC **(Table 3).**

Table 3.

Studies of histologically or cytologically verified BCC with 3- or 5-year follow-up after topical PDT

Study	Design	n	P R	Subtype	Lesion preparation	Treatment	Follow-up (years)	CR rate (%)
Basset-Seguin[161]	Prospective RCT	60	P	sBCC	Sligth debride-ment	MAL-PDT	5	75
Rhodes[135]	Prospective RCT	53	P	nBCC	Curettage	MAL-PDT	5	76
Souza[160]	Prospective	15	P R	sBCC nBCC	Non	DMSO-EDTA ALA-PDT	5	64
Star[151]	Prospective	86	P	sBCC	Salicylic acid 10 %	ALA-PDT	Mean 5	84
Mosterd[134]	Prospective RCT	85	P	nBCC	Debulking	ALA-PDT	3	70
Solér[107]	Retrospective	350	P R	sBCC nBCC	Curettage/ debulking	MAL-PDT	Mean 3	79
Baptista[152]	Prospective	70	P	sBCC nBCC	Non	ALA-PDT	Mean 3	71

RCT = randomized controlled trial, P = primary BCC, R = recurrent BCC, sBCC = superficial BCC, nBCC = nodular BCC, CR = complete response, n = number of BCC treated with topical PDT

(Provided by Christensen E.)

Both ALA- and MAL-PDT are proved to be effective in BCC.[87,112] There is little published clinical data comparing ALA- and MAL-PDT, but in a small, randomized study of nodular BCCs, no significant difference in efficacy was found.[94] A direct comparison of various study results is difficult because of differences in study design with use of different topical photosensitizes, light sources, treatment sessions, pre-treatment and inclusion of different BCC subtypes. In addition, different definitions of treatment failure yield different results. This point is clearly demonstrated with the disparity between complete response rates found in the 6- and 10-year follow-up, despite the fact that no new cases of treatment failure was noted during the last 4 years of the 10-year study.

In the treatment of BCC, curettage can be combined with other modalities such as surgery, electrodessication and cryosurgery, to delineate tumour margins and to reduce tumour thickness.[153-155] Curettage may also be used alone.[156] In Papers III and IV, a

particularly high complete response rate of at least 90% was shown in primary BCCs receiving two PDT-sessions. The pre-PDT preparation may have contributed to this outcome. This preparation included debulking of thicker tumours. The purpose of debulking was not to remove all parts of the tumour, only to remove the main tumour bulk within clinical margins. The pre-PDT preparation also included curettage of the perilesional area to minimize the risk of recurrence from subclinical BCC extensions.[157,158]

The use of DMSO may have further contributed to the favourable outcome by initiating ALA-induced porphyrin production[122] and by enhancing ALA-PDT penetration in the BCC.[119] A favourable short-term treatment outcome has previously been demonstrated in a study using DMSO-EDTA-ALA-PDT and two different light sources.[159] The 6-month complete response rate in superficial BCC was more than 80% in both groups. In another study using DMSO-ALA-PDT in nodular BCC, the mean 17-month response rate was 95% in those BCCs having a clinical complete response 3 to 6 months after intervention. However, a complete response rate of only 64% was demonstrated in a 5-year follow-up study after DMSO-EDTA-ALA-PDT.[160] In this study, both recurrent and nodular BCC were included, no pre-PDT preparation was performed and only one treatment session was given.

Results after treatment with various standardized therapies are also difficult to compare with PDT results, because of the lack of uniformity in study design such as inclusion criteria and different treatment protocols.[64] Setting this point aside, the high and sustained PDT efficacy demonstrated in Papers III and IV, particularly in BCCs having received two PDT sessions, is comparable with long-term results after various non-MMS treatments for this type of skin cancer.[63,66] Five-year recurrence rates following excisional surgery, curettage with electrodessication, radiotherapy and cryotherapy are reported to be between 5 to 10%, 8 to 13%, 4 to 16% and 8 to 26%, respectively.[58,63,66,161] Overall five-year treatment response of superficial BCC following imiquimod cream 5% is reported to be about 80%.[162,163]

The cumulative recurrence rate following surgery, radiotherapy and curettage and electrodessication in BCC also show that 50% of recurrences are identified within 2 years and 66% are diagnosed within the first 3 years of treatment.[63] After use of topical

PDT, it appears that the majority of recurrences occur during the first years. In Paper IV, 87% of recurrences appeared within 2 years and no further recurrences were observed beyond 3 years of follow-up. This observation is consistent with results from other long-term follow-up PDT studies that, with few exceptions, report recurrence of BCC to present itself within 3 years after treatment.[134,135,160,161]

A randomized, controlled study design is preferred when analysing treatment effects. A few randomized studies comparing topical PDT efficacy with other traditional BCC therapies have been published.[134,135,161,164-167] With the exception of one study, surgery was more effective than PDT, but no significant difference between cryosurgery and PDT was found. No difference in treatment efficacy after 12 months was observed when comparing ALA-PDT with cryotherapy in mixed BCCs in an early study from 2001.[164] A similar result was later found with curettage and MAL-PDT; the 5-year complete lesion response rate in the MAL-PDT group was estimated to 75% compared to 74% in the cryotherapy group.[161] Five other studies report on follow-up results after treatment with either topical PDT or excisional surgery of BCC. The first study demonstrated a 5-year sustained lesion cure-rate for surgery of 96% compared with 76% for MAL-PDT.[135] The second found a 3-year treatment failure of 2% for surgery compared to 30% for ALA-PDT.[134] In the third study, small superficial BCCs were treated. This study showed a recurrence rate of approximately 9% in the MAL-PDT group and no recurrence in the surgery group after 12 months.[165] The fourth study reported 38% treatment failure in the ALA-PDT group and 21% failure in the surgery group 12 months after treatment of nodular BCC.[166] In the fifth study, ALA-PDT was demonstrated to be as effective as excision surgery after treatment of 94 superficial and nodular BCCs with a mean follow-up period of 25 months.[167]
In addition, PDT has been shown to be more effective in the treatment of nodular BCC than placebo. Two multicentre, randomized, double-blinded studies comparing MAL-PDT with placebo cream, found treatment response rates of 73 and 27%, respectively.[168]

Treatment efficacy is one among several factors that determine the choice of therapy for the treatment of BCC. As BCC often arise in cosmetically or functionally important areas, many clinical considerations and patient's needs should be taken into account when deciding on treatment method.[73,78,169,170] PDT has shown long-term superior

cosmetic outcome when compared to treatment outcome by more invasive treatment modalities.[134,135,161,164] The prospect of better cosmetic outcome has been reported to influence patient preference in favour of PDT over surgical excision for treatment of BCC.[170] Cosmetic outcome has in previous studies been rated as good or excellent from 87 to 100% at 3 and 5 years after PDT.[107,135,160,161] The attractive post-treatment cosmetic results demonstrated in Papers III and IV add evidence to these results.

Three-month clinical examination of treatment areas after PDT is reported to overestimate the response compared with histology.[118,164,171] The clinical complete response rates are previously shown to range from 87 to 95% compared to histological results ranging from 50 to 85%. On the other hand, histological investigation of biopsy tissue from treated areas, clinically evaluated to be in complete response at 12 and 32 months after PDT, appeared to be of limited value (Papers III and IV). By use of histology, only one additional remnant BCC was revealed that was missed by clinical investigation. Further, even though the histology 12 months after treatment was negative, three recurrent BCCs appeared at a later time. It cannot be ruled out that the punch biopsies were taken from areas that were not representative of the residual tumour, as the histopathological specimens were obtained using 2 or 3 mm punches that necessarily can not sample the whole area of the original BCC. The benefit of possibly detecting a few recurrent tumors earlier by histological investigation rather than by clinical examination of the treatment areas should be weighted against the drawback of taking multiple biopsies as part of the follow-up procedures. The present study results and knowledge of BCC to be slow growing and with extremely low potential to metastasize, suggest that clinical examination of the treatment areas suffice for long-term follow-up. Patients should, however, be encouraged to perform self-examination and be advised to consult a medical doctor if they notice changes in treated areas.

Various patient- and lesion-related factors are reported to be associated with treatment failure.[58,78] Factors that might be associated with failure after PDT were explored in Papers III and IV. Some patients presented with more than one BCC. In Paper III, initially all lesions were handled independently as traditional statistical methods require that observations are independent. This strategy was chosen in order to utilize all available information, and because multiple BCCs within the same patient

often were of various subtypes and with different body location. However, research has provided an increasing knowledge of anti-tumour immune response after PDT. The direct damage of abnormal cells initiates a variety of the host's response strategies, such as inflammation and activation of innate and adaptive immunity that may affect the results.[86] As a consequence, the data were re-analyzed. When using a robust variance model that takes clustering of lesions into account, the association of gender and H-zone with treatment failure was even more significant than the first reported values.

Treatment failures were more frequent in men than in women. This has not, to our knowledge, been found in previous PDT studies reporting on gender. Data on sex differences in BCC are scarce, but evidence show that men have a higher incidence of BCC than women.[3,172,173] Also, men have been known to have deeper invasion of tumours, dissimilar tumour localisations, and men are more prone to develop BCC in response to other factors than women.[20,34,173,174] Some studies report higher BCC recurrence in men than women after MMS and radiotherapy.[175,176] It has been speculated that more prominent male hair follicles may contribute to deeper BCC growth into tissue.[34]

Patients' age and use of immunosuppressive medication were factors not found to be related to an increased risk of BCC relapse. This is in line with a few other PDT studies reporting on treatment failure in relation to patient's age.[108,134] However, in a recent Danish publication, patients over the age of 60 had significantly higher recurrence rates compared to younger patients.[177] Publications on PDT efficacy in the treatment of BCC in immunosuppressed patients are scarce. The short-term remission rate in a study of 32 (mainly BCC) facial tumours treated with PDT is shown to be 75%.[178] However, in a recent MAL-PDT study of 18 organ-transplant recipients, Guleng and Helsing[179] achieved a BCC recurrence rate of only 6% with a mean follow-up of 22.6 months. Recurrent lesions (failures of previous treatment) are considered to be high risk lesions and are often of the histological aggressive types.[2] PDT is not regarded as a first line treatment of such lesions.[58,180] As many as three out of five recurrent BCCs did not completely respond to PDT within the 3 first months (Papers III and IV). Also in a recent PDT 3-year follow-up study the cure rate for recurrent BCC was low, estimated to 63%.[181] Interestingly, after treatment with PDT with a mean follow-up of 22 months,

Solér et al.[182] achieved a cure rate of 82% in BCCs that previously had recurred after radiotherapy. However, a pre-PDT skin shaving procedure and up to five treatment sessions were performed in this study.

It is suggested that BCC lesions located within the H-zone are generally more difficult to treat, regardless of method, due to the potential of BCC growth into unexpected depths along embryonic fusion planes.[79,158,183,184] However, this theory has not been proven. BCC location within the H-zone was found to be associated with recurrence of tumour after PDT in Paper III, but not in Paper IV. This is an illustrative example of how much an apparently small change in analyses strategy can affect the results. With topical PDT in BCC, several studies have failed to demonstrate a difference in treatment response by site.[108,134,165,185] PDT has even been shown to be most effective for facial lesions, as reported in a study by Foley et al. using minor debulking and MAL-PDT in nodular BCC. On the other hand, Solér et al. reported a complete response rate of 76% in BCCs located to the H-zone compared to a rate of 92% for tumours located outside this zone, in a curettage and MAL-PDT study of a total of 350 superficial and nodular BCCs.[107] Of concern is that the H-zone area appears to be defined somewhat differently as it is marked with slight variation on figure illustrations in different publications.[62,78,105,183] Differences across studies whether a lesion is inside or outside this zone can occur and may have affected the various results.

In Papers III and IV, the majority of BCCs were considered to be small (≤ 2 cm), thus the conclusion only applies to small lesions. The only lesion larger than 2 cm resulted in treatment failure. There is evidence indicating that larger BCC have higher recurrence than smaller lesions after PDT, but the size limit varies in the different studies.[161,168,171,184]

BCC thickness or subtype was not found to be associated with treatment failure after PDT (Papers III and IV). In the long-term follow-up results, we demonstrated a complete response rate of 82% in tumours more than 2 mm thick. Only a few studies have investigated the influence of BCC thickness on PDT outcome, and the results returned from these studies are ambiguous.[107,108,134,168] In a study by Morton et al.[185], BCC thickness was showed to affect treatment response significantly. ALA-PDT was performed without pre-treatment preparation of the treatment area. No tumours with

thickness more than 2.0 mm showed complete response. In a recent study by Fantini et al.[108] using MAL-PDT in BCC, tumour thickness was also shown to have an impact on treatment response, with lower cure rates with increasing tumour thickness. The overall complete response was 62%; 33% for nodular BCC, but no tumour debulking was performed. In another study, comparing curettage and MAL-PDT with placebo and curettage, treatment response in the PDT group proved slightly less effective in BCCs with baseline thickness more than 1.0 mm. Nevertheless, 82% complete response of tumours measuring from 2.0 to 5.0 mm thick was reported.[168] In a curettage and ALA-PDT study of thick nodular BCCs, no significant difference of BCC recurrence was found according to whether tumours were thinner or thicker than 1.3 mm.[134] The comment presented by the authors with regard to this finding, was that the thickness measurements from a biopsy specimen might not be representative for tumour thickness of the entire lesion. This view is supported by the findings in Paper II.

Furthermore, pre-PDT curettage and debulking may significantly reduce BCC thickness.[124] Thus, the pre-treatment preparation should be described in detail as this procedure may have a great influence on the treatment outcome. This information is often either lacking or insufficiently described in many publications. Information on BCC thickness is usually based on histological examination of a diagnostic biopsy specimen. However, a pre-treatment biopsy may not represent the BCC thickness at the time of treatment. Thus the pre-treatment thickness measurement may be misleading in the assessment of PDT effect with regard to BCC thickness. This caution also applies to results in Papers III and IV.

Different histological methods for BCC subtype classification were used in Papers I-IV. In Paper III, tumours were classified into superficial or nodular types, based on growth down to or below the papillary dermis.[129] Such a classification is currently rarely in use as it only provides information about the growth-depth and not the morphologic growth type. In Paper IV, BCC were classified into aggressive and non-aggressive subtypes, based on histologic growth pattern.[31] No significant association was found between treatment failure and subtype, defined by any of these two classification methods. Although histological examination is the basis for subgroup analyses, the information provided from a biopsy can still be limited. According to the

literature, punch biopsies are accurate in predicting BCC subtype in only 69 to 89% of cases.[186-188]

CONCLUSIONS

- Cytological skin scrape diagnosis for BCC was dependable.
- There was no significant difference in sensitivity between Pap and MGG stain for skin scrape cytodiagnosis in BCC.
- Touch imprint cytology was not found useful as a routine diagnostic method.
- Reasonable overall agreement was found between mean (average) BCC tumour thickness in punch biopsy and excision specimens.
- Predicting BCC thickness from a single punch biopsy of individual lesions could be inaccurate: increasing with increasing BCC thickness.
- High treatment efficacy and favourable cosmetic outcome was achieved 6 years after two sessions of topical DMSO-ALA-PDT in BCCs that showed complete clinical cure 3 months after treatment.
- All BCC recurrences appeared within 3 years after treatment.
- Recurrent BCC, male gender, one treatment session and lesion location within the H- zone were factors associated with treatment failure after topical PDT.
- Two sessions of curettage and topical DMSO-ALA-PDT gave a 10-year high and sustained efficacy with a favourable cosmetic outcome in small and primary BCCs.

CLINICAL IMPLICATIONS

Given the magnitude of BCC, the need for better diagnostic and therapeutic approaches is continuous.

Skin scrape cytology with either Pap or MGG stain, is a diagnostic method to be considered for confirmation of clinically suspected superficial BCCs suitable for non-invasive treatment modalities such as topical PDT, and of clinically suspected BCC relapses following PDT. A high degree of representative cell material can be obtained by careful scraping the surface of the lesion with a small curette after removal of the keratotic surface. Cytodiagnosis can also be performed on cell material obtained as part of the PDT pre-treatment procedure when no prior histological diagnosis is available.

Prior knowledge on tumour thickness is important for selection of BCCs suitable for topical PDT. BCC thickness measurements from single 3 mm punch biopsy specimens may not be representative of the thickness of the whole tumour, and thus insufficient for planning of therapy in some cases. With a punch biopsy tumour thickness measurement of 2 mm or more, the BCC is thicker than the current recommended limit for topical PDT, according to the international PDT consensus guideline. A measurement of 1 mm or less suggests that the tumour most likely will be within the recommended limit for PDT. However, with a measurement between 1 and 2 mm the true tumour thickness may well exceed the current recommendation. In such cases the choice of therapy may be reconsidered. Should the indication for PDT be compelling, despite uncertainty with regard to tumour thickness, supplementary biopsies may be taken before treatment and/or a close follow-up after PDT may be prescribed.

Two sessions of curettage and topical DMSO-ALA-PDT give a high treatment efficacy in selected primary, small BCCs and with a favourable cosmetic outcome. Most failures occur within 3 years follow-up. Based on our studies, histopathological examination of specimens from small punch biopsies from treated areas in clinical complete response is of limited value in long-term follow-up.

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ERRATA

The flow chart (Figure 1) in Paper III contains misprints. The number of patients with lesions showing partial treatment response at 3 months should be n = 4 and the number of patients with recurrence at 36 months should be n = 1. These mistakes propagate further in the chart. However, the numbers of lesions in the chart are correct. The second last “assessment box” below 24 months should read 36 months.

Paper I

Is not included due to copyright

Paper II

Acta Derm Venereol 2011; 91: 651–655

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.***

(Accepted February 2, 2011.)

Acta Derm Venereol 2011; 91: 651–655.

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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.

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).

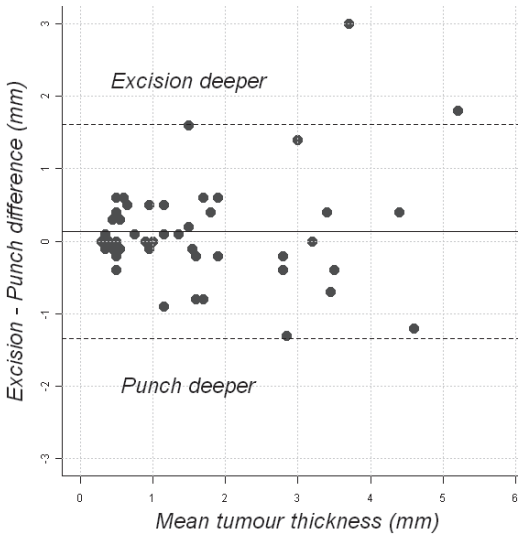

Fig. 1. Bland-Altman plot showing difference between measurements by excision and punch biopsy, according to mean tumour thickness. The mean difference (bias) was 0.14 mm, with 95% limits of agreement at –1.3 and 1.6 mm.

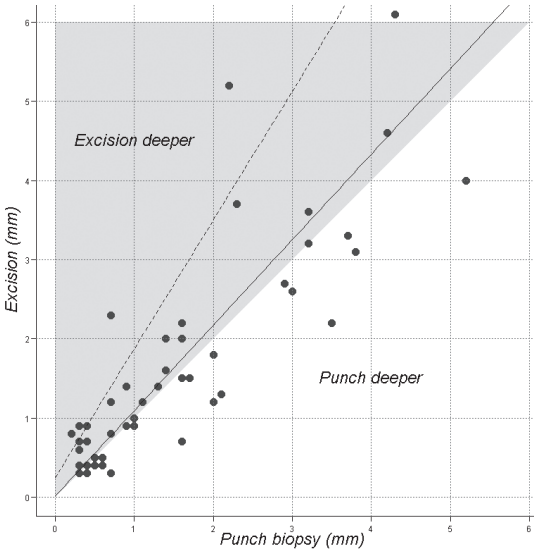

Fig. 2. Relationship between measurements of tumour thickness in corresponding punch biopsy and excision specimens. The shaded, grey area shows where excision depth exceeds punch biopsy depth with the line of identity at the border. The mean (——) and 95% upper (- - -) prediction lines are superimposed.

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.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Øystein Grimstad for his contribution to the study, Professor Cato Mørk for review of the paper and Dr Beti Evans, Cambridge, UK, for linguistic help in preparing the manuscript.

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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Paper III

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Paper IV

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High and sustained efficacy after two sessions of topical 5-aminolaevulinic acid photodynamic therapy for basal cell carcinoma: a prospective, clinical and histological 10-year follow-up study

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Summary

Background Prolonged follow-up data on topical photodynamic therapy (PDT) in basal cell carcinoma (BCC) are necessary for a full evaluation of its effect and for comparison with conventional treatment methods.

Objectives To assess 10-year long-term PDT efficacy in primary and recurrent BCC and to evaluate clinical and histopathological factors which may be associated with treatment failure.

Methods We performed a longitudinal study on 60 histologically verified BCCs in 44 patients treated with curettage and one or two sessions of dimethylsulphoxide (DMSO)-supported topical 5-aminolaevulinic acid (ALA)-based PDT. Treated lesions were investigated by clinical and histopathological examination at regular intervals. The main outcomes were 10-year lesion complete response rate using a time-to-event analysis, histological treatment failure and cosmesis.

Results Overall complete response rate for all lesions was 75% (95% confidence interval 64–87%); 60% after one and 87% after two treatment sessions. The response rate was 78% for primary lesions; 63% after one and 90% after two sessions. The cosmetic outcome was rated as good or excellent in 91–100% of evaluated cases. Treatment failure was documented in 15 (25%) of 60 lesions; clinical investigation identified 14 of them. All failures were noted within 3 years of treatment. Male gender, recurrent tumour and one treatment session were factors significantly associated with treatment failure. The only lesion larger than 2·0 cm relapsed.

Conclusions Two sessions of DMSO-supported topical ALA-PDT and curettage can provide long-term effective treatment results with favourable cosmetic outcome in primary, small BCC.

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Accepted for publication
25 January 2012

Funding sources
The work was not supported by external funding.

Conflicts of interest
E.C. has received reimbursement for attending medical meetings by Photocure/Galderma. C.M. has obtained lecture fees from Photocure/Galderma. E.S. has no conflict of interest to declare.

DOI 10.1111/j.1365-2133.2012.10878.x

Basal cell carcinoma (BCC) is the most common cancer in white people and its incidence is rising.[1] It most often appears on sun-exposed skin and can cause considerable local tissue destruction and patient morbidity.[2] Surgery and radiotherapy appear to be most effective among a variety of interventions available to treat BCC, with Mohs micrographic surgery showing the lowest failure rates.[3] Topical photodynamic therapy (PDT) is an attractive and minimally invasive treatment option in cases where surgery is considered suboptimal: in patients with multiple comorbidities, high risk of postsurgical scarring with or without functional impairment, large lesions, or where cosmetic outcome is of major concern.[4,5]

The most commonly used topical precursors of photosensitive porphyrins for PDT in BCC are 5-aminolaevulinic acid (ALA) and methyl aminolaevulinate (MAL).[6] PDT exerts its action through light activation of the photosensitizer in the

presence of oxygen, leading to the release of reactive oxygen species that cause a selective destruction of the targeted atypical cells.[7]

Since the pioneer publication by Kennedy *et al.* in 1990[8] several studies have provided information on the effect of topical PDT,[6,9] with 5-year data showing long-term clearance rates from 64% to 86% in BCC.[5,10,11] Several strategies have been explored to enhance the treatment effectiveness, particularly when treating thicker tumours, as this therapy has limited skin-penetrating abilities.[12,13] The use of dimethylsulphoxide (DMSO), which is a drug penetration enhancer, and the use of pretreatment lesion preparation can improve treatment efficacy.[12,14]

Using these strategies we have previously reported 6-year ALA-PDT follow-up data showing an overall tumour clearance rate of 81% among those lesions clinically observed to be in complete response 3 months following treatment.[15]

Although this result adds to evidence supporting topical PDT to be an effective treatment modality, several studies have shown that results achieved using different treatment methods will vary depending on the length of follow-up time.[16–19] With various traditional types of treatment, evidence suggests that up to 18% of recurrent BCCs present clinically between 5 and 10 years following therapy.[16] Prolonged follow-up data are thus necessary for a full evaluation of topical PDT as a treatment alternative to conventional modalities for BCC.

The main objective of the present study was to assess 10-year long-term treatment response and cosmetic outcome of primary and recurrent BCC after DMSO-supported topical ALA-PDT and curettage. A secondary aim was to explore clinical and histopathological factors that may be associated with treatment failure.

Materials and methods

Patients and lesions

Patients referred to the Department of Dermatology, St Olav's University Hospital, Trondheim from 1 September 1997 to 1 September 1998 with suspected BCCs were screened. Excluded from treatment by topical PDT were patients under the age of 18 years, those who where pregnant or breastfeeding, or those with Gorlin syndrome.

Excluded from the study were lesions clinically evaluated as morphoeic or pigmented, lesions that had been treated within the last 6 months, where the diagnostic biopsy was older than 6 months, or lesions with thickness in excess of 3·5 mm by histology. Lesions without histologically verified diagnosis in advance of treatment, or lesions that received more than two PDT treatment sessions were also excluded.

The localization of all lesions was marked on a chart, and lesions were photographed for later identification of treatment sites. Lesion size was estimated clinically as the mean value of the greatest length and width and classified into three groups: $< 1{\cdot}0$, $1{\cdot}0–2{\cdot}0$ and $> 2{\cdot}0$ cm.

The study was approved by the regional committee for medical research ethics (4.2008.2877) and patients gave written informed consent.

Treatment procedure

From September 1997, two treatment sessions of curettage and topical ALA-based PDT were performed 1–2 weeks apart. From February/March 1998, only one treatment session was given.

Both physical and chemical PDT supportive measures were used to enhance penetration of ALA into tumour tissue. All lesions were pretreated with superficial curettage with the intention only to remove the upper part of epidermis on visible tumour and about 4 mm beyond its margin. In addition, careful intratumoral curettage (debulking) to reduce tumour volume was performed occasionally, depending on clinical assessment of tumour thickness. No local anaesthesia was used. Following curettage, a small piece of gauze soaked in DMSO 99% (Merck, Darmstadt, Germany) was applied to the lesions located on trunk and extremities for 5 min. DMSO is a chemical solvent which disrupts the skin barrier. An emulsion of 20% ALA (Norsk Hydro, Oslo, Norway) and Unguentum Merck (Merck, Hermal, Reinbek, Germany) was then applied on to the treatment site. On lesions located on face and hairy scalp DMSO 4% was added to the ALA-containing emulsion.

The treatment area was covered by a semipermeable dressing. Residual emulsion was removed after 3 h in accordance with former Norwegian ALA-PDT experience. The treatment area was then exposed to light for about 8–10 min. A broadband halogen light source (Instrumentation Department, Norwegian Radium Hospital, Oslo, Norway) was used, with an emission spectrum of 550–700 nm and a light intensity of 150–230 mW cm^{-2} at the lesion surface.

Follow-up procedures

The post-treatment follow-up period was initially intended to be 1 year, and was thereafter gradually prolonged. Patients were followed up by dermatologists at the outpatient clinic at 3, 6, 12, 24, 36, 72 and 120 months after treatment. The treated sites were evaluated by inspection and palpation and classified as either complete response or as treatment failures. Treatment failure comprised both lesions with initial 3-month noncomplete response and lesions with recurrence of tumour within or contiguous to the treatment area. Clinically suspected treatment failures were confirmed by histology. In addition, all lesions in complete clinical remission were biopsied for histological examination at 12- and 36-month follow-up. A 2 or 3 mm punch biopsy was used to obtain tissue samples in all cases.

The cosmetic outcome was assessed by the investigator at 6, 12, 24, 36, 72 and 120 months and by the patients at 24, 36 and 72 months after therapy. The outcome was recorded on a four-point ordinal scale as excellent (absence of any stigmata other

than scar formation after diagnostic punch biopsy) or as good, fair or poor (presence of fibrosis, atrophy or change of pigmentation, judged as slight, moderate or marked, respectively).

Histology

Pathologists at the Department of Pathology and Medical Genetics, St Olav's Hospital examined the diagnostic biopsy specimens, which were routinely processed and stained with haematoxylin, eosin and saffron. Tumour thickness was measured from below the stratum corneum to the base of the tumour nest on the histologically prepared sections and classified into three groups: < 1·0, 1·0–2·0 and > 2·0 mm. Retrospectively, the histopathological sections from the diagnostic punch biopsies were re-examined by one pathologist. The tumours were subclassified into nonaggressive and aggressive growth types based on their histopathological growth pattern. The nonaggressive category included the superficial and nodular BCC types and the aggressive category included the micronodular, morphoeiform and infiltrative types.

Data presentation and statistical analysis

Descriptive data are reported as numbers and/or percentages. Treatment failure or 'cure' (i.e. no treatment failure) was first tabulated according to relevant predictor variables disregarding the time of recurrence, and analysed with χ^2 tests. Second, by taking time into consideration, treatment failures were plotted along the time axis as Kaplan–Meier plots; the steps were drawn at the time when tumour recurrence was noted and registered by the dermatologist.

Univariate analysis was done using the log rank test, and a Cox proportional hazard model was fitted using a backward approach until only significant predictors were retained in the model. In the Cox model, the event time for detection of treatment failure was set to midway between the previous (no recurrence noted) and the follow-up visit when recurrence of tumour was identified. The analysis was adjusted for clustering of lesions within patients. $P < 0{\cdot}05$ was considered statistically significant. Analysis was done using the package *survival* using the statistical software R version 2.12.[20]

Results

Forty-four patients (22 men, 26 lesions; 22 women, 34 lesions) with 60 BCC lesions were followed. The mean age at entry was 72 years (range 35–91). A variable number of patients attended the scheduled appointments, but all patients except those who died and those who had experienced treatment failure were examined at the 72- and 120-month follow-up visits (Fig. 1).

Thirty-four patients had one lesion, eight patients had two and two patients presented each with five lesions. Five lesions represented recurrences after previous treatments: four after surgery and one after cryotherapy. The mean size was 0·7 cm (range 0·2–2·1); only one lesion was larger than 2·0 cm. Of 35 lesions located to face, head and neck area, 31 were < 1·0 cm and the remaining four were < 1·5 cm in size. Twenty-five lesions received one treatment session and 35 lesions were treated twice. Further information on lesion baseline clinical and histopathological characteristics is shown in Table 1.

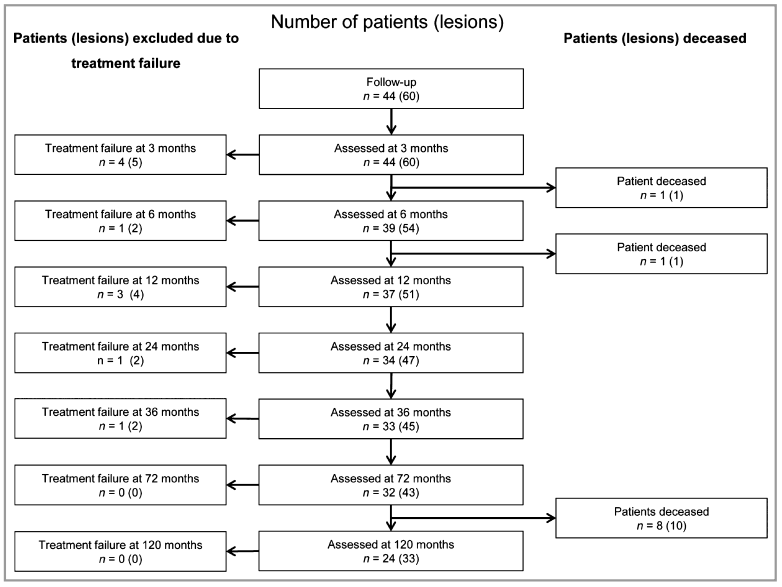

Fig 1. Flow of patients and lesions.

Table 1 Lesion baseline characteristics and lesion response to photodynamic therapy

	Baseline		Treatment failure		
Characteristics	All lesions (n = 60), n (%)	Primary lesions (n = 55), n (%)	All lesions (n = 15), n (%)	Primary lesions (n = 12), n (%)	P-value[a]
Gender					
Male	26 (43)	22 (40)	11 (42)	8 (36)	0·016
Female	34 (57)	33 (60)	4 (12)	4 (12)	
Age (years)					
≤ 71	30 (50)	30 (55)	6 (20)	6 (20)	0·55
> 71	30 (50)	25 (45)	9 (30)	6 (24)	
Immune status					
Immunocompetent	54 (90)	50 (91)	13 (24)	11 (20)	0·79
Immunosuppressed	6 (10)	5 (9)	2 (33)	1 (20)	
Treatment sessions					
1 session	25 (42)	24 (44)	10 (40)	9 (38)	0·036
2 sessions	35 (58)	31 (56)	5 (14)	3 (10)	
Light dose (J cm^{-2})					
75	46 (77)	43 (78)	12 (26)	10 (23)	1
100	14 (23)	12 (22)	3 (21)	2 (18)	
Size (cm)					
< 1·0	47 (78)	44 (80)	10 (21)	9 (20)	0·16
1·0–2·0	12 (20)	10 (18)	4 (30)	2 (20)	
> 2·0	1 (2)	1 (2)	1 (100)	1 (100)	
Location of tumour					
Face, head, neck	35 (58)	31 (56)	11 (31)	9 (29)	0·23
Trunk	19 (32)	18 (33)	4 (21)	3 (16)	
Extremities	6 (10)	6 (11)	0	0	
H-zone					
Inside H-zone	22 (37)	20 (36)	7 (32)	7 (35)	0·54
Outside H-zone	38 (63)	35 (64)	8 (21)	5 (14)	
Histological pattern					
Superficial	14 (24)	14 (26)	2 (14)	2 (17)	0·47
Nodular	28 (47)	25 (46)	9 (32)	7 (28)	
Aggressive	17 (29)	15 (28)	4 (24)	3 (25)	
Lesions included, n	59	54			
Tumour thickness (mm)					
< 1·0	27 (47)	27 (51)	5 (19)	5 (19)	0·23
1·0–2·0	20 (34)	15 (28)	8 (40)	5 (33)	
> 2·0	11 (19)	11 (21)	2 (18)	2 (18)	
Lesions included, n	58	53			

All lesions = primary and recurrent lesions. [a]The P-values refer to χ^2 tests of the cross-classification of the given characteristics and outcome among all lesions (e.g. for gender: 11 failures out of 26 in men vs. four out of 34 in women, P = 0·016).

Treatment evaluation

Ten years following PDT the overall BCC complete response rate was 75% [95% confidence interval (CI) 64–87%]. The rate was 60% after one treatment and 87% after two treatment sessions (Fig. 2). For primary lesions only, the response rate was 78% (95% CI 68–90%); 63% after one and 90% after two treatment sessions.

Treatment failure was documented in 15 of the 60 (25%) treated lesions; in 10 of 25 (40%) after one and in 5 of 35 (14%) after two treatment sessions. BCC was identified at the scheduled follow-up visits at 3, 6, 12, 24 and 36 months in 5, 2, 4, 2 and 2 treated sites, respectively. Histopathological examination revealed one case of tumour residue that was not captured by clinical investigation at 12 months follow-up. The cosmetic outcome was rated as excellent or good by the investigator in 91%, 97%, 97%, 100% and 100% of cases at the follow-up visits at 12, 24, 36, 72 and 120 months, respectively. The patients rated the cosmetic outcome as excellent or good in all cases.

In the log-rank univariate analyses, the clinical factors male gender (P = 0·0046), recurrent tumour (P = 0·0047) and number of treatment sessions (P = 0·0317) were associated with treatment failure. Similar results were obtained from the multivariate Cox model (male gender P = 0·020, recurrent tumour P = 0·00024, treatment sessions P = 0·0030). Treatment failure was identified among men in 11 of 26 (42%) lesions and among women in 4 of 34 (12%) lesions

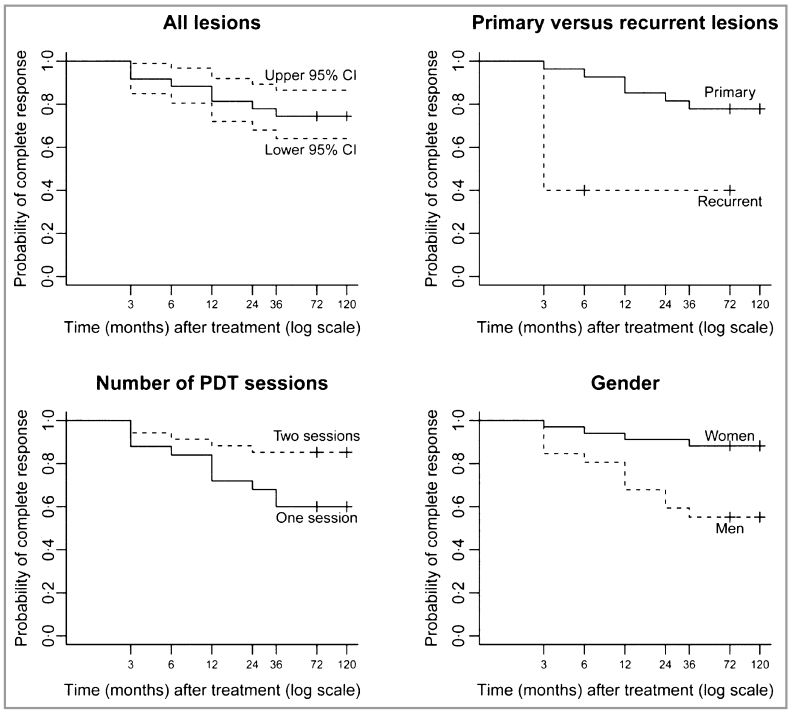

Fig 2. Probability of lesion complete response over time. The 'steps' are drawn at the visit when recurrence was noted by the treating dermatologist. CI, confidence interval; PDT, photodynamic therapy.

(Table 1). Of the five recurrent tumours included, treatment failure was observed in three (60%) within 3 months following PDT. None of the other clinical or histopathological factors was found to be significantly associated with treatment failure (Table 1).

Discussion

The present study demonstrated a high and sustained 10-year PDT efficacy of 87% in BCC receiving two treatment sessions, and a 90% complete response rate shown in primary tumours. This is comparable with long-term results after conventional nonsurgical therapies.[16,19,21] However, it is difficult to compare different therapies because of lack of uniformity in study design such as inclusion criteria and different statistical methods.[21]

In the present study, for best approximation of true response rates, a time-to-event approach was chosen to estimate lesion response rate over time.[19] Treatment outcome was assessed at the follow-up visits and recorded either as complete response or treatment failure. The reported response rates include the residual tumours we observed 3 months after treatment that in many other PDT studies are not counted as failures.[22]

At follow-up, histopathological investigation revealed treatment failure in only one case that was missed by clinical assessment. We cannot rule out that sampling error may be a problem as histopathological specimens were obtained using small (2 or 3 mm) punches not necessarily representative for the complete lesion.

Treatment efficacy was significantly higher in primary lesions receiving two treatment sessions compared with those receiving only one. This finding supports the current accepted practice of two PDT sessions in the treatment of BCC.[6,9] However, as the lesions (patients) were not randomized with respect to the given number of treatment sessions, this result should be evaluated with caution as selection bias cannot be ruled out. One may still argue that this allocation was 'random', in the sense that it depended on when treatment was given: early or mid-way through the treatment period. Guidelines on optimal number of PDT treatment sessions were not available in 1997. The initial practice of using two treatment sessions when the method was first introduced to our department was later reduced to one session, as we obtained more experience with the technique.

The present study reflects 'real life' in the sense that few exclusion criteria were used and even recurrent (failure of previous treatment) lesions were included. Recurrent BCCs are known to have an increased risk of relapse, with 5-year long-term recurrence rates between 10% and 40% after treatment with various non-Mohs modalities.[17] These are more often of

the histological aggressive subtype and considered high-risk tumours.[2] Reports on topical PDT efficacy of recurrent BCC are scarce. However, in a 3-year follow-up after topical PDT of 11 recurrent BCCs the cure rate was estimated to 63%.[23] In the present study the cure rate did not exceed 40% but only five lesions were evaluated. Noninvasive therapeutic methods are currently not regarded as a first-line treatment for these cases.[17]

Apart from our 6-year follow-up study of the same cohort, we are not aware of other studies suggesting that men have a greater propensity for recurrence of BCC after PDT.

BCC incidence is reported to be higher in men than in women,[1,3,24] male gender is found to be predictive of extensive subclinical tumour spread,[25] and BCCs in men have been reported to possess a propensity towards deeper invasion than those in women.[26] Some studies found that men have an increased tendency to experience tumour recurrence than women after Mohs micrographic surgery or radiotherapy.[27,28] The reason for these findings has not been established. BCC is a follicular tumour and it has been speculated that the more prominent male hair follicles may contribute to deeper BCC growth into tissue.[26]

Several BCC characteristics are known to be associated with higher recurrence rates including tumour thickness, subtype, size and location, particularly to the mid-face area (H-zone).[22,29] In the present study no significant association was found between recurrence and these factors. PDT for treatment of > 2·0 mm thick BCC and for aggressive growth-type tumours achieved a good outcome compared with the results in thinner lesions and in tumours considered to be of a more low-risk growth type. Most lesions were small. The only lesion larger than 2·0 cm resulted in treatment failure, but no firm conclusion based on this single finding can be made. A higher percentage of tumours within the H-zone recurred compared with tumours located to face, head and neck area outside this zone, but the difference was not statistically significant. However, the size of the study population limits the value of subgroup analyses.

In a systematic review by Rowe *et al.* the cumulative recurrence rate following surgery, radiotherapy and curettage and desiccation in BCC showed 50% of recurrences within 2 years and 66% appearing within the first 3 years of treatment.[16] In addition, 18% of recurrences were found to appear as late as between the 5th and 10th year.

Current long-term follow-up data after PDT also demonstrate that BCC recurrence rate increases with prolonged follow-up time: no-recurrence reported to be 93–97% at 3 months, decreasing to 64–81% at 5–6 years following treatment.[5,10,11,15] However, it appears that most recurrences after PDT occur during the first years after treatment. In a randomized study comparing MAL-PDT with surgery with 5-year follow-up, more than 80% of treatment failures appeared within 2 years and no recurrence of BCC was noted after 3 years follow-up.[5] Similar results were found at 5-year follow-up of a randomized study comparing PDT using MAL with cryotherapy. Most treatment failures were identified within the first 2 years after PDT and no recurrences were observed after 3 years in the PDT group.[10] Most tumour recurrences were also detected within 2 years following treatment in a randomized study comparing ALA-PDT with surgery in 173 nodular BCCs. In this long-term study, all recurrences except one appeared within 3 years after PDT.[30] Also in a smaller ALA-PDT based study treating 15 BCCs, three of four treatment failures appeared within 3 years after treatment and the fourth appeared between the 3- and 6-year follow-up visits.[11] In the present study, as many as 87% of all treatment failures appeared within 2 years and no failures were observed beyond 3 years following treatment.

Both topical ALA- and MAL-PDT are proved to be effective in BCC.[6,9] Despite MAL being more selectively taken up into tumour tissue than ALA,[31] there is no evidence, at present, that MAL-PDT is more efficient in the treatment of nonmelanoma skin cancer. However, PDT procedures are currently not standardized, making an exact comparison of results between various studies difficult.

The present study demonstrated a particular high cure rate in primary tumours receiving two treatment sessions. The use of DMSO may have contributed to the favourable result. DMSO has been shown to initiate ALA-induced porphyrin production[32] and to enhance ALA-PDT effect in BCC.[33] Pretreatment lesion preparation may further have contributed to the favourable outcome, by also including the perilesional area to minimize the risk of recurrence from subclinical tumour extensions.[34] In accordance with our national PDT practical guidelines,[35] this curettage was aimed at gently removing the upper epidermis to facilitate the skin penetration of ALA without compromising the cosmetic outcome. This was rated as excellent or good in 100% of all evaluated cases at both the 6- and 10-year visits.

In conclusion, two sessions of curettage and DMSO-supported topical ALA-PDT provide a long-term effective and cosmetically favourable outcome in primary, small BCC. Most treatment failures appear within 2 years of treatment.

What's already known about this topic?

- Evidence suggests that treatment results will vary depending on the length of follow-up time, and up to 18% of recurrent basal cell carcinomas (BCCs) occur 5–10 years following conventional treatments.

What does this study add?

- The outcome after two sessions of topical dimethylsulphoxide-supported 5-aminolaevulinic acid-based photodynamic therapy and curettage, showing a 10-year cure rate of 90% in primary, small BCCs, with all recurrences appearing within 3 years of treatment, is comparable with results of other well-accepted nonsurgical therapies.

Acknowledgments

The authors thank Dr Trond Warloe for his contribution to the study design and Dr Patricia Mjønes for assistance with histopathological investigations. We also thank Dr Anna M. Bofin for reviewing the manuscript.

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