Fluorescence Detection and Diagnosis of Non-Melanoma Skin Cancer at an Early Stage

Jaap de Leeuw, MD,^{1,2}* Nick van der Beek, Msc, LLM,^{1,3} W. Dieter Neugebauer, MD, PhD,^{1,4} Peter Bjerring, MD, PhD,⁵ and H.A. Martino Neumann, MD, PhD²

¹ZBC MultiCare, Outpatient Clinic for Dermatology and Laser Therapy, 1217 AB Hilversum, Netherlands

²Department of Dermatology, Erasmus MC, Erasmus University, 3000 CA Rotterdam, Netherlands

³Utrecht University, Utrecht 3512 BL, Netherlands

⁴Euromed Clinic, 90763 Fürth, Germany

⁵Faculty of Medicine, Swansea University, SA2 8PP Swansea, Wales, United Kingdom

Background: The occurrence of non-melanoma skin cancer (NMSC), including actinic keratosis (AK) is increasing all over the world. The detection and diagnosis of NMSC is not optimal in clinical practice. Complementary methods for detection and accurate demarcation of NMSC at an early stage are needed in order to limit the damage caused by tumours.

Objective: The purpose of the present study was to use a large area skin fluorescence detection system to detect early NMSCs (clinical visible as well as non-visible lesions) in the face, neck, chest, back and hands of patients treated with UV and outdoor workers.

Methods: Fluorescence detection with a purpose-made digital camera and software (Dyaderm[®]) combined with 5-aminolevulinic acid (5-ALA) encapsulated in liposomes.

Results: In 93 consecutively referred patients positive skin fluorescence was detected in 61 patients. After histological examination the positive fluorescence appeared to be correlated to benign lesions in 28 patients (sebaceous gland hyperplasia in 22 patients) and to (pre-) malignant lesions in 33 patients (actinic keratosis in 29, BCC in 3 and SCC in 1 patient). False negative fluorescence was found in only one lesion. In five patients the FD technique used in this study appeared to be more sensitive for the identification of (pre-) malignant lesions than the clinical examination. This is in contrast with FD techniques used in previous studies.

Conclusion: Diagnostic skin fluorescence using liposomal encapsulated 5-ALA and a specialised computerised detection and visualisation system offers the possibility for detection of NMSC at an early, pre-clinical stage. The technique is well suited to examine large areas of skin. It also identifies areas of most interest for performing confirmatory skin biopsies, as well as pre-operative assessment of boundaries of skin malignancies, and finally, the technique is applicable in the control and follow-up of skin cancer treatment. Lasers Surg. Med. 41:96–103, 2009. © 2009 Wiley-Liss, Inc.

Key words: actinic keratosis; 5-aminolevulinic acid; fluorescence detection; liposomes; skin cancer

INTRODUCTION

Non-melanoma skin cancer (NMSC) is the most common cancer in Caucasian populations and its incidence is of epidemic proportions worldwide [1-5]. Although, the term NMSC covers all cutaneous cancers excluding melanomas, it is normally used to refer to two major types of skin cancer: basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). These two forms of skin cancer account for more than 95% of all NMSC [5]. BCCs arise de novo, which means there are no known precursor lesions [5]. SCCs may arise from actinic keratoses (AKs). Often, these lesions have been considered to be pre-malignant precursors [6-8], but according to other authors, AKs are true epithelial neoplasmata from the beginning [3,9]. From this point of view, the evolution from AK to SCC represents progression rather than transformation, and therefore, AK should be recognised as incipient SCC [10,11]. It has been estimated that 6-10% of AKs develop into invasive SCC [12,13]. This highlights the value of AK as a marker for SCC [3]. Thus, if these high-risk patients are monitored closely, invasive tumours that develop could be treated at an early stage when cure rates are high, morbidity is low, mortality is nonexistent, and costs of treatment are limited [3]. The average annual increase of NMSC in white populations in Europe, the United States, Canada and Australia has been 3-8% since the 1960s [4]. Nowadays, NMSC incidence is greater than all other cancers combined and NMSC is among the five most costly cancers reported in the USA [14]. There are many reasons for the worldwide increase in NMSC incidence. Etiological factors that underlie the development of skin cancer are of endogenous origin as well as of exogenous origin [4]. Major endogenous factors are age, genetic pre-disposition, such as skin type (in particular skin types I, II and III) and genetic diseases (e.g. xeroderma

^{*}Correspondence to: Jaap de Leeuw, MD, Department of Dermatology & Venereology, Erasmus MC, University Medical Center, P.O. Box 2040, 3000 CA Rotterdam, Netherlands. E-mail: jaapdeleeuw@hetnet.nl

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pigmentosa). The key environmental risk factor for NMSC is ultraviolet (UV) light radiation from exposure to sunlight, artificial tanning lamps [15-18] and iatrogenic exposure to psoralen and ultraviolet A (PUVA), and to ultraviolet B (UVB), modalities often used for the treatment of psoriasis and vitiligo [19,20]. Persons with occupational or recreational outdoors exposure, such as commercial fisherman, construction workers, park rangers, farmers, aircraft pilots, yachtsmen, golfers, skiers, mountaineers and sun-addicts, have higher incidence rates of AKs than indoor living people [21-24]. Other exogenous factors include chemical carcinogens (such as arsenic, pesticides, tar, certain industrial oils, dves and solvents), ionising radiation, human papilloma viral infections [25] and immunosuppression, especially in organ transplant recipients [26]. Tobacco smoking has also been recently linked to SCC [27]. In 2002, more than 1 million cases of NMSC were diagnosed in the United States [28]. SCC accounts for more than 20% of NMSC and most metastatic disease and death due to NMSC. Therefore the ability of the physician to distinguish pre-cancerous lesions with a high degree of accuracy is of great importance and will decrease the morbidity and mortality of SCC [29]. The clinical features of AKs are non-specific, usually described as red, scaly papules or plaques, 2–10 mm in diameter, occurring in sun-exposed sites. They may bleed and may become hypertrophic, at which point a biopsy should be performed to evaluate the progression to SCC [29]. The differential diagnosis of AK includes many benign and malignant lesions (Table 1) [13,29]. This broad differential underscores the highly non-specific clinical features of AKs [29]. The diagnostic accuracy or positive predictive value (PPV) of the clinical diagnosis of AKs made by specialised doctors appeared to be only 74% after histological diagnosis [29]. In particular field cancerisation, in which there are multiple (pre)malignant lesions diffusely spread over UV-exposed skin, is an increasing problem. Up till now, physicians often need to take multiple biopsy specimens of the clinically malignant-suspect lesions, which can be a significant burden for the patient [30]. A sound public health policy adapted to the challenges of the 21st century must strive to prevent skin cancer development through risk factor modification (primary prevention) and improved disease surveillance and earlier detection (secondary prevention) [31]. There is a need for more complementary methods for detection and accurate demarcation of skin cancers at an early stage, in order to limit the damage caused by tumours [30, 32].

 TABLE 1. Differential Diagnosis of Actinic Keratosis

| Benign | Malignant |
|---------------------------------------------------------|-------------------------|
| Keratosis (seborrheic, stucco, arsenical, lichenoid) | Bowen's disease |
| Porokeratosis | Basal cell carcinoma |
| Keratoacanthoma | Squamous cell carcinoma |
| Lentigo solaris | |
| Discoid lupus erythematodes | |
| Psoriasis | |

The mechanism of 5-aminolevulinic acid (5-ALA) used in photodynamic therapy (PDT) is based on intra-cellular transformation of 5-ALA to protoporphyrin IX (PpIX), followed by light exposure, which induces phototoxicity. Besides its usefulness in PDT, 5-ALA and methylaminolevulinic cream (MAL, Metvix[®], Galderma) also can be utilized for diagnostic purposes. The transformation of 5-ALA can be monitored non-invasively by detection of PpIX fluorescence at 634 nm. Fluorescence from the skin after application of 5-ALA is the result of a complex interaction of 5-ALA penetration into the skin [33,34], cellular absorption of 5-ALA [33-35], metabolic transformation of 5-ALA into PpIX [36] and the clearance time of 5-ALA and PpIX [33,34]. For topical application of 5-ALA relatively high concentrations of 5-ALA are necessary, because of the low rate of penetration of free 5-ALA into the skin. Generic preparations of 5-ALA 20% in a moisturising cream (pharmacy preparations) or methylaminolevulinate (MAL) 16% cream (Metvix[®] Galderma) are used under an impermeable plastic occlusion film to enhance and standardise absorption. Following treatment with this high concentration preparations photosensitisation remains for at least 32 hours. Exposure to ambient light within this time interval may cause severe clinical signs of phototoxicity, for example swelling, erythema and scaling. Therefore, patients receiving this type of treatment are advised to avoid bright light and sun exposure for 24-48 hours after treatment [37].

Liposomes are microscopic vesicles consisting of concentric bilayers formed by phospholipids, enclosing an aqueous core. Therefore, liposomes contain lipophilic compartments within the bi-layer membranes and hydrophilic compartments between the membranes. Lipophilic substances can be stored into the lipid phase and water soluble substances into the water phase. Therefore, liposomes can be used as carriers for lipophilic as well as hydrophilic drugs [38]. Three mechanisms have been described concerning the penetration of liposomes into the epidermis: penetration via the pilosebaceous units [39], via lateral diffusion of liposomes in the stratum corneum [40], and via a transepidermal osmotic gradient and hydration force [41]. The similarity of lipid composition of liposomes and epidermis enables the liposomes (and also the drugs encapsulated in them) to penetrate into the epidermal barrier to a greater extent than lotions, creams and ointments do [42]. In addition, liposomes bind to cells and deliver their contents to the cytoplasm of cells by fusion with the outer cell membrane or by endocytosis, upon which they are concentrated in lysosomal sacs [43]. These capabilities make liposomes very useful for the enhancement of the penetration of lipophilic as well as hydrophilic drugs (5-ALA is hydrophilic) into the epidermis [44]. An optimal penetration of liposomes requires a dry skin surface. Under occlusion (maximal hydration) no penetration of liposomes occurs [41].

The fluorescence of skin areas treated for more than 1 hour with 20% ALA cream under occlusion appears to be very uniform and the fluorescence intensity continues to increase after the end of 5-ALA application. The maximum fluorescence is reached at 8 hours after the end of 20% 5-ALA application [37]. The fluorescence of skin areas following non-occluded topical application of liposomeencapsulated 0.5% 5-ALA was heterogeneously distributed (heterogeneous distribution shows high fluorescence intensity from diseased tissue and low fluorescence intensity from normal skin) and reached a saturation level after 2 hours. The fluorescence decays linearly within 15 minutes after the end of application of 0.5% 5-ALA liposomes and returned to baseline within 8 hours [37]. The average skin surface fluorescence induced by the liposome-encapsulated 0.5% 5-ALA applied for longer than 2 hours, is found to be equal to the average measured skin surface fluorescence obtained after 30 minutes exposure to 20% 5-ALA cream [37]. Changing the 5-ALA vehicle from a moisturising cream to liposome encapsulation, the 5-ALA concentration can be lowered by a factor of 40, and still induce the same skin fluorescence and at the same time it eliminates the need for occlusion [37]. Following topical application of 5-ALA, PpIX is induced selectively in cells with high metabolism, such as in epithelial tumour cells. Upon irradiation with light the tumour becomes visible and can be delineated from the surrounding tissue [45]. This method is called fluorescence diagnosis (FD). By using a highly light sensitive charged couple device (CCD) camera system together with a specially designed digital imaging (Dvaderm system, Biocam GmbH, Regensburg, Germany), the contrast of the acquired fluorescence images can be significantly enhanced [30,45,46]. This allows the determination of the optimal site for a directed biopsy and/or may indicate the boundaries of the tumour for pre-operative planning when Mohs' surgical treatment is scheduled [45]. Moreover, FD is also a helpful tool to evaluate the efficacy of PDT [45]. Although promising, the results till now were limited due to technological constraints both in terms of application of 5-ALA, illumination and observation of fluorescence [47]. The current technology deviates from preceding methods in three ways. Firstly, the previously used flashlamp is replaced by a homogeneous LED light source. Secondly, the 5%-ALA in a moisturising cream under occlusion is replaced by liposomal encapsulated 5-ALA 0.5%, without occlusion. Thirdly, the traditional FD systems with their relatively limited area are replaced by a specialised version of the Dyaderm[®] system. This new system enables investigation of large skin areas in one picture, resulting in less time consuming procedures. The aim of this study was to establish FD, using liposomal encapsulated 5-ALA and a specialised version of the Dyaderm[®] system, as a reliable guide for the detection of NMSCs (including AKs). An additional objective was to asses the diagnostic efficacy of heterogeneous fluorescence following application of 5-ALA 0.5% liposomes without occlusion in contrast to homogeneous fluorescence from 16% 5-methylaminolevulinic cream (MAL, Metivix[©], Galderma) under occlusion.

MATERIALS AND METHODS

Patients

The study was conducted on a consecutively recruited group of 93 persons with a history of chronic UV exposure, such as outdoor professions and long-term UV therapy, or with a history of treatments for NMSC. The group consisted of 43 females and 50 males with an average age of 59 years (standard deviation 11 years). Pregnancy, recent peeling or scrubbing or other rejuvenation techniques of the skin, and allergy to 5-ALA were exclusion criteria. The study areas were limited to highly UV exposed areas such as the face, the scalp, the chest, the upper back and the back of the hands. Prior to the FD procedure, the test areas of the skin were degreased by wiping with gauze moistened with acetone.

5-ALA Preparation

In the present study an emulsion was used containing 0.5% 5-ALA encapsulated in 50 nm sized unilamellar liposomes, which are the preferred structures for drug delivery [38]. The liposomal solution was prepared according to a pharmacy-protocol from Dianorm GmbH (Munich, Germany). The preparation is chemically stable for at least 24 months. The concentration of 0.5% 5-ALA appeared to be sufficient for fluorescence detection, meanwhile strongly reducing the risk of post-procedure phototoxicity [37].

The only known side effect of 5-ALA liposomal preparation is that it may induce a slight erythema and scaling when applied to skin which has recently been treated with a peeling or scrubbing agent or skin which is very dry where the epidermal barrier function is not intact. Attention was given to these potential side-effects during application.

Fluorescence Detection System

In this study fluorescence measurements were performed with a specially designed version of the DyaDerm[®] fluorescence detection system, which is a highly sensitive digital fluorescence imaging system intended for the analysis of larger skin areas. Each image dimension is 14 by 18 cm, giving an imaging skin size of 252 cm². A LED pulsed light source is used, which emits blue light with a peak wavelength of 405 nm. Pulse duration is 5 milliseconds and repetition frequency is 1 Hz. The intensity of each LED is 1.0 W. This light source is common for the version of the Dyaderm[®] system used in this research. The resulting fluorescence signal is recorded by a 10 bit CCD camera mounted to an adjustable stand and coupled to a computer system where image acquisition and processing is controlled by the DyaDerm[®] software. Because of the short pulsed excitation photobleaching of PpIX is minimised. As PpIX fluorescence emission consists of light in the red spectrum, the red pixels of the CCD camera are used to generate a fluorescence image. Blue light also activates other fluorophores in the skin, such as lipopigments and flavins, which emit light in the green spectrum. By comparing the red light resulting from the red fluorescence of PpIX and the (green) auto-fluorescence from the lipopigments and the flavins an accurate and detailed image of the lesions is created, including a relative measurement of fluorescence intensity on a cardinal ranking, making intrapatient fluorescence comparisons possible. By computing the relative intensities of the PpIX and auto-fluorescence image, inhomogeneities in the fluorescence intensity due to

imperfections of the excitation light field and inhomogeneities due to the curvature of the observed object are automatically corrected. The resulting image is referred to as "PpIX filtered" and is also presentable in pseudocolour (Fig. 1a,c) [48]. The DyaDerm[®] system can capture and display colour images of the skin and their corresponding fluorescence images in a live video mode while artefacts due to camera motion are suppressed by using a fixed stand. These images can be superimposed and pseudo-coloured to better highlight relevant parts of increased fluorescence due to malignant or pre-malignant lesions. Because images are captured in video mode using both white and blue conditions, the system is triggered to capture every fifth image under fluorescent light. In this way, both colour and fluorescence images are captured and transferred to the display. After interpolation, both images can be superimposed to allow a physician to localise any high-fluorescence spots at the examined skin (Fig. 1b). It is possible to present the fluorescence intensity in pseudocolour. The human eye distinguishes colours better than different shades of brightness, thus with a pseudo-colour image a tumour is much easier to localise. In this pseudocolour representation a colour bar is shown at the bottom of the image. The distribution of colours over the relative fluorescence is linear. The blue colour represents the lowest value of relative fluorescence included in the image, the red colour represents the highest value of relative fluorescence, and all intermediate colours are linear distributed over the intermediate values of relative fluorescence. As mentioned the relative fluorescence is a ratio of the intensity of the red light induced by PpIX fluorescence and the green light induced by the autofluorescence. When using the system the physician can acquire the exact value of relative fluorescence of a certain region by hovering the mouse above that location, resulting in a pop-up bar showing the local value. In practice the system therefore offers more information than can be shown in this article [48].

These presentations support, amongst others, easy detection of sebaceous hyperplasia and AK's. Fluorescence image from sebaceous gland hyperplasia shows circumscribed, regularly defined fluorescence showing medium (yellow) to high (red) intensity (Fig. 3a). AKs are clearly visible with a well defined compact core of high intensity, shown in red, and a surrounding halo of intermediate fluorescence levels which is also well defined with borders sharply demarcated against the normal tissue (Fig. 3b,c).

Comparison of the Fluorescence Properties of 5-ALA 0.5% Liposomal Spray With 5-Methylaminolevulinic 16% Cream (MAL, Metvix[©], Galderma)

MAL16% cream was applied under occlusion on the right side of the forehead for 3 hours. Liposomal 5-ALA 0.5% spray was applied every 5 minutes on the left side of the forehead without occlusion for $2\frac{1}{2}$ hours, followed by a pause of $\frac{1}{2}$ hour to allow the 5-ALA liposomes to be absorbed completely into the skin. During the application of the spray, the patients resided in a room with dimmed light. Immediately after this, fluorescence pictures were made of both sides of the forehead.

Establishment of the Efficacy of FD, Using Liposomal Encapsulated 5-ALA and a Specialised Version of the Dyaderm System for the Detection of NMSC (Including AK)

After admission the patients were situated in a room with dimmed light where they applied the 5-ALA 0.5% spray every 5 minutes for $2\frac{1}{2}$ hours to the involved skin area. Thereafter the spray was left to be absorbed into the skin for an additional half hour. Although the intensity of the blue pulsed light emitted from the fluorescence detection system is not harmful, theoretically it may trigger a seizure in patients with epilepsy. Therefore, patients who either suffered from epilepsy or had a close relative suffering from epilepsy were examined wearing dark eye goggles. Subsequently, pictures were taken using the fluorescence detection system. In order to minimise any disturbance from ambient light, the pictures were taken in a completely darkened room. The pictures were evaluated by a trained dermatologist. The general threshold level for demarcating tumour from normal skin (fluorescence ratio) was found to be 1.37 times the mean value of the fluorescence in the ALA treated area using 4 hours of ALA cream application [49]. In this study skin areas showing a red centre surrounded by a yellow halo (intensity of fluorescence in the red centre above 1.5 compared with the fluorescence of the surrounding normal skin) were considered to have a positive fluorescence pattern. From these skin areas of



Fig. 1. The sequence of images from the skin of a female patient with a histologically confirmed basal cell carcinoma on the upper back: **a**. Colour image from the upper back, **b**. Fluorescence image showing the skin tumor position, **c**. Processed image, where in pseudo-color the tumor position, known from the fluorescence image, is superimposed and blended into the original image. Irregularly defined fluorescence showing medium (yellow) intensity.



Fig. 2. **a**. Fluorescence image right side of the forehead, 3 hours after application of MAL cream under occlusion: High fluorescence in most of the studied skin area, showing low discrimination between normal and diseased skin, **b**. Fluorescence image left side of the forehead 3 hours after application of 5-ALA 0.5% liposomal spray (every 5 minutes for $2\frac{1}{2}$ hours and $\frac{1}{2}$ hour more for optimal absorption of 5-ALA into the skin) without occlusion: Low fluorescence from the

most interest biopsies were taken under local anaesthesia with 1% lidocaine-adrenalin for histological diagnosis.

Ethics

The study protocol (number MC 2007-01) and associated information were submitted to and approved by an ethics committee prior to the study. All investigations are in accordance to all applicable legal norms and non-legal standards.

RESULTS

The fluorescence image of the right (MAL-treated) side of the forehead showed very high and homogeneous fluorescence intensity in most of the studied skin areas with low discrimination between normal and diseased skin (Fig. 2a). The left (ALA-liposome treated) side showed low autofluorescence of the normal skin and moderate, but distinct fluorescence of actinic keratoses, resulting in a high discrimination between the normal and the diseased skin (Fig. 2b). The 5-ALA-liposome solution seems to be highly superior to MAL for discrimination between normal skin and NMSC lesions (Fig. 2a,b). Moreover, the significantly lower uptake of 5-ALA in liposomes into the normal skin results in lesser degree of photosensitivity and -toxicity and the known faster clearance of 5-ALA ensures a much better safety profile of the 0.5% 5-ALA liposome preparation compared with MAL cream (8 vs. 36 hours of photosensitivity) (Fig. 2c).

normal skin in the studied skin area and distinct fluorescence from actinic keratoses, resulting in a high discrimination between normal and diseased skin, **c**. Fluorescence image forehead 36 hours after MAL cream right side and 36 hours after 5-ALA liposomal spray left side. The fluorescence of 5-ALA liposomal spray is of lower intensity and of lower duration compared to MAL cream.

A positively fluorescence intensity was detected from 287 lesions in 61 of the patients. After histological examination the positive fluorescence appeared to be correlated to 212 benign lesions in 28 of the patients and to 75 (pre-) malignant lesions in 33 of the patients (Table 2). The benign lesions were histological identified as sebaceous gland hyperplasia (204) in 22 patients (Fig. 3). Other benign lesions (eight) were found in six of the patients included viral warts, a benign lichenoid inflammation and dysplastic melanocytic nevi. The (pre-) malignant lesions were histologically diagnosed as actinic keratosis (71) in 29 of the patients. In four patients four malignant lesions were histologically confirmed to be three BCCs (Fig. 1) and one SCC.

False negative fluorescence was found in only one lesion (a negative result by fluorescence detection of a lesion clinically suspected and histological confirmed to be an AK). This lesion, situated on the vertex of the head was noted by the patient because of a thick hyperkeratosis and was clinically identified as actinic keratosis by the examining doctor.

In five of the patients AKs, not noted by the patients nor by the examining doctors were identified by fluorescence detection and later confirmed by histological investigation. This means that in contrast to previous studies [47] the number of tumours assessed by the FD technique used in this study was higher than the number of tumours determined by clinical diagnosis.



Fig. 3. **a**. Fluorescence image from subaceous gland hyperplasia: Circumscribed, regularly defined fluorescence showing medium (yellow) to high (red) intensity, **b**. Fluorescence image from a solitary actinic keratosis: Irregular defined fluorescence, showing medium (yellow) and high (red) intensity, **c**. Fluorescence image from multiple actinic keratoses: Irregular defined fluorescence showing medium (yellow) to high (red) intensity.

| | Number of lesions | % of lesions | Number of patients | % of patients |
|-----------------------------|-------------------|--------------|--------------------|---------------|
| Total 93 patients | | | | |
| No fluorescence | 0 | 0 | 32 | 34.3 |
| Positive fluorescence | 287 | 100 | 61 | 65.7 |
| Sebaceous gland hyperplasia | 204 | 71.1 | 22 | 23.7 |
| Other benign lesions | 8 | 2.8 | 6 | 6.5 |
| Actinic keratosis | 71 | 24.7 | 29 | 31.2 |
| Malignant lesions | 4 | 1.4 | 4 | 4.3 |

TABLE 2. Classification of the Results

DISCUSSION

In order to obtain a good fluorescent demarcation of tumours by FD it is important that the fluorescence contrast between the tumour and the normal skin is as high as possible [32]. The fluorescence from skin areas treated with 16% ALA cream under occlusion is very uniform (Fig. 2a), and hence there is only very low discrimination between normal and diseased skin. The fluorescence from skin areas treated with non-occluded liposome-encapsulated 0.5% 5-ALA is heterogeneously distributed, allowing for a high discrimination between normal and diseased skin (Figs. 1c, 2b and 3b,c) [37]. Moreover, the lower uptake of 5-ALA-liposomes into the normal skin results in a lower risk of post-procedure phototoxicity and the faster clearance of 5-ALA from the tissues results in a shorter duration of this phototoxicity compared with MAL cream (8 vs. 36 hours) (Fig. 2c). This means, that patients are less restricted from outdoor activities following application of 5-ALA-liposomes compared with MAL cream.

In this study liposomal encapsulated 5-ALA and the DyaDerm[®] fluorescence detection system were used to detect positive fluorescence in 61 patients. After histological examination the positive fluorescence appeared to be correlated to benign lesions in 28 patients and to (pre-) malignant lesions 33 patients (Table 2). The benign lesions were histologically identified as sebaceous gland hyperplasia 22 patients (Fig. 3a). This is not surprising, because sebaceous hyperplasia is a benign lesion characteristic of UV damaged skin and liposomes penetrate very well into the sebaceous glands [50]. The benign lesions displaying positive fluorescence could easily be identified as sebaceous gland hyperplasia on clinical examination because the sebaceous glands become clearly visible as yellow-white translucent papules when the skin is stretched. The clinical diagnosis of the aforementioned benign lesions can be confirmed by dermatoscopy. Under the dermatoscope sebaceous gland hyperplasia is characterised by the cumulus sign, crown vessels and horny pseudocysts [51], viral warts by hyperkeratosis and tiny petechiae and dysplastic melanocytic nevi by a melanocyte dermatoscopy score between 4.75 and 5.45 [52]. Of all the benign lesions with positive fluorescence only the lichenoid inflammation was not identified by clinical investigation and dermatoscopy. The combination of the fluorescence detection system used in this study with clinical investigation and dermatoscopy the specificity of this method appeared to be 92%.

The positive fluorescence of the benign lesions (viral warts, a lichenoid inflammation and dysplastic melanocytic nevi) indicates, that thickness and pigmentation of the epidermis does not considerably interfere with 5-ALA-liposomal FD. This is in contrast with a negative relation shown between the fluorescence emitted from the skin and the thickness of the epidermis using 5-ALA in a cream base [30].

The fluorescence image of the false negative result showed no distinct discrimination between the lesion and the surrounding skin, very probably caused by the abundant fluorescence of the surrounding hairs. This makes it likely, that FD is not an appropriate method to examine skin lesions situated on dense hairy sites of the body. Interference may also occur from dermal infections, viral warts and inflammatory diseases, like psoriasis. Taken the false negative result into account the sensitivity of the described method was established to be 97%.

This new non-invasive technique is time saving for the doctor, because it can be performed by auxiliary personnel presenting the physician with images which can be assessed at a glance. It is advisable to apply the current system in a professional environment. A custom robotic arm can be used during the fluorescence detection operation and a totally darkened room is needed. In addition, special rooms with dimmed light where the patients can apply the 5-ALA spray are required. This makes the system less suitable for the daily practice in a general hospital. The creation of specialised detection centres would make sense, especially given the large population which are at risk of acquiring a NMSC.

Future studies may optimise the correlation between fluorescence patterns and clinical and histological pictures, especially concerning the discrimination of penetrating from non-penetrating malignancies.

CONCLUSIONS

In the field of fluorescence detection 5-ALA-liposomes appear to be superior to MAL cream for discrimination between normal skin, benign lesions and NMSC lesions. Moreover, 5-ALA-liposomes are safer to use due to lesserand shorter duration of post-procedure phototoxicity compared with MAL cream.

A substantial percentage (23.7%) of the patients appeared to have positive fluorescence caused by sebaceous gland hyperplasia. Because sebaceous gland hyperplasia can easily be identified on clinical examination and by dermatoscopy they did not interfere in the detection of DE LEEUW ET AL.

NMSC. Interference may also occur from dermal infections, viral warts and inflammatory diseases, like psoriasis.

The FD technique used in this study appeared to be more sensitive for the identification of (pre-) malignant lesions than clinical examination in contrast to the fluorescence techniques used in previous studies [42].

False negative fluorescence was found in only one lesion situated on the hairy scalp of the patient, indicating that FD may not to be an appropriate method for examining skin lesions situated on dense hairy sites of the body.

FD using liposomal encapsulated 5-ALA and a specialised version of the DyaDerm[®] system offers the possibility for detection of NMSC at an early, pre-clinical stage and the technique can easily examine large skin areas skin. It also identifies areas of most interest for performing confirmatory skin biopsies, as well as pre-operative assessment of boundaries of skin malignancies, and finally the technique is well suited for control of treatment.

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