PHOTODYNAMIC THERAPY AND FLUORESCENCE DIAGNOSIS OF NON- MELANOMA SKIN CANCER

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INTRODUCTION

1.1 Non-melanoma skin cancer

Epithelial skin cancers, also called non-melanoma skin cancers (NMSCs), are the most commonly occurring skin tumors, primarily affecting the Caucasian race, especially people with Fitzpatrick I or II skin type. The most common NMSCs are actinic keratosis (AK), basal cell carcinoma (BCC), Bowen's disease (BD) and invasive squamous cell carcinoma (SCC). NMSC patients have a higher risk of a second skin cancer. Although mortality from NMSC is low, disability and disfigurement may result from these cancers**.** The presence of actinic keratoses may be regarded as a major warning sign that subclinical cellular damage has been accumulated. The subclinical field changes mean the widespread presence of DNA-damaged cells which are called "field cancerization".

The gold standard in the treatment of non-melanoma skin cancer is surgical excision, but several alternatives exist, especially for superficial lesions. Considering the potential presence of subclinical lesions, just treating visible lesions does not solve the overall carcinogenic risk. Treatment possibilities which target DNA-damaged cells give a more forward-looking approach to treating sun damaged skin and prevent tumor progression. An optimal cosmetic result is often given a higher priority than total removal due to the slow growth and low metastatic rate of these tumors.

1.2 Photodynamic therapy and fluorescence diagnosis

PDT has recently become accepted for the treatment of non-melanoma skin cancer. The essence of PDT is that the introduction of an exogenous photosensitizer into the skin cells results in the accumulation of protoporphyrin IX (PPIX) in rapidly proliferating cells. During therapy, the skin surface is illuminated at an appropriate wavelength, which leads to the excitation of PPIX. As the PPIX returns to the basic energetic state, reactive oxygen radicals are generated, and these induce the apoptosis of tumor cells. Topically applied photosensitizers, like 5- or delta-aminolevulinic acid (ALA) or its methyl ester the methyl-aminolevulinate (MAL), by themselves do not cause sensitivity to light. However, they metabolize in the cells, thus resulting in the formation of photosensitising protoporphyrin IX in the process of heme synthesis. While ALA enters cells mainly through active transport, MAL, possessing lipophilic properties, can also enter cells through passive transport. In choosing the light source used during PDT two important factors must be kept in mind: 1. the

applied light must reach the layer of skin which is to be treated, 2. its wavelength must line up with the peaks of the absorption spectrum of PPIX. The penetration of light into the skin is directly proportional to the wavelength - blue light absorption is the most superficial, red and infrared penetrates most deeply. On the absorption curve of the PPIX the highest peak is at 405 nm, this wavelength corresponds to blue light emitting light sources. Further absorption peaks can be found at 510, 545, 580, 630 nm. On the absorption curve of the PPIX the highest peak does not belong to the 630-nm wavelength light – the most commonly used wavelength in the oncodermatological practice, but red light penetrates deeper than the shorter wavelength blue or green light.

Limiting factors of PDT include thick tumours (> 2 mm), and the pigment content of tumours (melanin pigment absorbs light). PDT is contraindicated in morpheic BCC. Side-effects of PDT are erythema, crusting, serous discharge, oedema, developing several hours after the treatment. Therapy-related pain is the most frequent side-effect. Patients usually report a cumulative burning sensation during illumination that becomes intense within a few minutes after the start of the procedure. In some cases, the pain may become so severe that the illumination must be stopped prematurely, with the result that the applied light dose, and the PPIX formation are insufficient, and the therapeutic result is inadequate.

Topical PDT is a highly effective mode of treatment in AK, superficial BCC and BD. Prospective, randomized studies have proven that the efficiency of PDT was similar to surgical excision, similar or better than cryotherapy and 5-FU, however its cosmetic outcome is far more superior. Accordingly, PDT is currently regarded as first line treatment in case of AK, superficial BCC and BD. In non-melanoma skin cancers, MAL and ALA are equally effective as photosensitizers in PDT.

PPIX accumulated in epithelial cells may be detected with the help of ultraviolet light by its fluorescence. On UV imaging the coral red fluorescence outlines the border of the damaged or neoplastic area. Thus fluorescence diagnosis (FD) allows an accurate assessment of tumor borders and may be used on one hand to perform guided biopsy, on the other hand to plan the lines of excision prior to surgical removal**.** Furthermore, it may help at follow-up after PDT in deciding whether the repetition of the treatment is necessary when the response to therapy is difficult to judge. At the beginning of the incubation the rapidly proliferating, photo-damaged or malignant cells with rapid metabolism take up the porphyrin precursor, the difference between the detectable fluorescence of the neoplastic area and the surrounding healthy area is increasing. However with extended incubation time also normal cells take up the porphyrin precursor. At this moment the fluorescence ratio between tumor and intact

tissue is decreasing again. For photodynamic diagnosis (PDD) and therapy generally a 3-hour photosenzitizing period is used.

Mohs micrographic surgery (MMS) is especially used for the treatment of high-risk basal cell carcinoma located on the ear and the middle of the face. Tumors located in these areas often threaten the integrity of eyelids, nose cartilages, lips, as their size cannot always be judged clinically. Since lobe rotation or skin graft transplantation is often needed it is of utmost importance that the excision is done within healthy tissue to avoid the need for reexcision. This is why an accurate determination of the edges of the lesion is crucial. Mohs micrographic surgery increases the effectiveness of the excision in healthy tissue, however, it is time-consuming, cumbersome and expensive. With the help of FD the extent of the tumor and its edges can be judged much more accurately. This technique reduces the number of excisions in Mohs microsurgery thus shortens the surgical time.

At a follow-up visit the most accepted method to judge the effectiveness of treatment and the occurrence of recurrence was done physically (visual, both dermoscopic and tactile). Photodynamic fluorescence enabling to determine the actual size of the tumor helps in giving a more accurate assessment of the therapeutic response and recurrence. If FD gives positive result at the follow up visit, PDT can be made easily.

1.3 Diagnosis of non-melanoma skin cancers with in vivo reflectance confocal microscopy

The in vivo reflectance confocal microscopy (RCM) technique allows examination of the superficial areas of the skin, the epidermis and the upper part of the dermis with high resolution images. Various skin lesions can be examined with the confocal laser scanning microscope in vivo, "real time" mode or they can be tracked with the help of standard recordings taken at different occasions. This way we are able to get a picture of the skin which can even be similar to histomorphology without being invasive. In certain cases, biopsy can be avoided with this technique, which is particularly important when we plan for a non-invasive treatment promising a better cosmetic result than standard therapy, like in the case of photodynamic therapy of AK, sBCC or BD. Since the thickness of skin lesions suitable for PDT usually coincides with the domain which we can examine using a RCM device (0-200 μm), the method is suitable for examining these premalignant skin lesions and tumors before and after treatment. Based on the literature, actinic keratosis and Bowen disease with in vivo reflectance confocal microscope can be diagnosed based upon the following criteria: in the area of stratum corneum, disruption, parakeratosis, and hyperkeratosis is characteristic. In the

area of the stratum granulosum-spinosum architectural disarray (keratinocytes with irregular shape and size, irregular honeycomb pattern) and cellular nuclear pleomorphism, exocytosis, spongiosis are visible. The upper dermal area is characterized by solar elastosis, increased vascularisation, and the presence of inflammatory infiltration. BCC has a typical RCM image also. The nuclei and cells elongate, so they become monomorphic and they become polarized. This appears partly as longitudinal bundle-like arrangement, partly as palisade ordering on the edge of tumor nests. Lobulated tumor cell-nests can be detected in batches which depending on the pigment content may appear with extent reflectivity (light islands of tumor cells) or may be dark (dark silhouettes). Around them slit-like, dark zone can be seen.

2. AIMS

I. The aim of our study was to evaluate the degree of treatment-associated pain during PDT with two different photosensitizers, ALA and MAL, in different anatomical regions, consideration being given to differences in diagnosis, age, gender and the method of pain relief.

II. Our goals were to supplement the traditional physical examination of epithelial skin tumors before PDT with other non-invasive diagnostic methods like in vivo reflectance confocal microscopy and fluorescence diagnostics. We wanted to examine the potential detectability of protoporphyrin IX accumulation using confocal microscopy and to monitor the changes seen in the tissue immediately after photodynamic therapy. Furthermore we intended to investigate the effectiveness of therapy using RCM and FD.

3. PATIENTS AND METHODS

3.1. Examination of pain during PDT with two different photosensitizers, ALA and MAL

During the 4 years between December 2003 and November 2007, PDT was performed on 182 occasions to treat NMSC in our Department. Eighty-seven patients were involved (32 females, 55 males, mean age=72 years, age range=43-92 years). The locations of the tumors were as follows: head and neck region: 111 (cheeks: 22, forehead: 31, temporal area: 12, nose: 18, auricular region: 12, lip: 1, scalp: 11, neck: 4), trunk: 45 (back: 29, chest: 15, abdomen: 1), and extremities: 26 (shoulders: 13, arms: 6, hands: 5, shin: 1, thigh: 1). PDT was performed on 80 occasions for AK, 97 occasions for BCC and 5 occasions for BD. Patients

were randomly assigned to receive either 20% ALA or 16% MAL (Metvix®, Galderma) ointment for 4 hours in an occlusive, light-reflecting dressing. ALA was used for 103 treatments (48 patients, 18 females, 30 males, age range=46-92 years, mean age=72 years) and MAL for 79 treatments (39 patients, 14 females, 25 males, age range= 43-87 years, mean age=70.4 years). 30 minutes prior to illumination, the patients were offered, and if requested, administered 500 mg paracetamol orally for pain relief. After photosensitizing, with the monochromatic diode lamp (Aktilite®, PhotoCure ASA, Oslo, Norway) situated 8 cm from the skin surface, illumination was performed with 630-nm visible red light at a dose of 37 J/cm^2. During the illumination, the skin surface was cooled periodically with wet gauze, if requested by the patient. The degree of patient-reported pain was assessed immediately after PDT on a 0-10 numeric rating scale (NRS), which was explained to each patient before the therapy (0 meaning no pain and 10 meaning unbearable pain).

One and two-way ANOVA, Student's T-test and the Scheffe test (*post hoc*), were applied for statistical analysis with Statistica 8.0 software (StatSoft Inc.). Scheffe's method was used to adjust significance levels for multiple comparisons in ANOVA.

Four weeks after the first treatment, a follow-up examination was performed to evaluate the necessity of further treatment. The tumors were rated into 3 groups from the aspect of the therapeutic result: complete remission (CR), incomplete remission (IR), or no response (NR) by an independent physician.

3.2. Examination of adjuvant methods for monitoring the efficacy of PDT

In our present study 12 patients (6 men, 6 women) were enrolled with whom PDT was planned in AK, sBCC and BD indications. The mean age of patients was 68.9 (age range 45-83 years). The lesions were distributed as follows: 4 actinic keratoses, 7 superficial basal cell carcinomas, 1 Bowen disease. With the help of an Olympus E-330 digital SLR camera and Clearstone ultraviolet digital imaging analysis system, normal and then UV photos were taken of the lesion that was to be treated. The clinical diagnosis was confirmed by a confocal microscopic examination prior to treatment (in 7 cases a biopsy were made also before treatment), the lesions showed typical signs appropriate for the three above mentioned diagnosis on the RCM image. When taking confocal microscopy images we used the VivaScope® 1500 Multilaser (MAVIG GmbH, Munich, Germany). The device operates at three wavelengths: 785 nm (near infrared), 658 nm (red) and 488 nm (blue). We investigated whether there was a difference between the images made by the three wavelengths and whether the photosensitizer ALA containing cream or PDT therapy would cause any change

on the RCM images. One selected actinic keratosis was recorded with all three lasers before applying the photosensitizer, immediately after removal of the cream and immediately after illumination. In the case of the 12 lesions, first we took a dermoscopic picture with the help of the camera of the selected lesion. After this we set the border of stratum granulosum-spinosum of the surface epithelium. From this point on we took 8x8 mm pictures in three layers every 25 microns. Then, according to our clinical practice we applied a 20% ALA photosensitizer ointment and an occlusive dressing to the skin for 3 hours. Subsequently, with the help of a Olympus E-330 digital SLR camera and Clearstone ultraviolet digital imaging analysis system, a UV photo was taken first. After the shooting, the tumor was illuminated with 630-nm visible red light at a dose of 37 J/cm^2 (Aktilite®, PhotoCure, LED light source). After illumination a third UV photo was taken and once again a confocal microscopy image recording was done in order to examine the immediate changes caused by the treatment. After 4 weeks we called our patients back for a follow-up visit. Then, in addition to physical examination, once again normal and UV photos were taken and in vivo confocal microscopy examination was performed.

4. RESULTS

4.1. Pain during photodynamic therapy

Immediately after PDT, the patients estimated the degree of pain felt during PDT on the scale 0-10; 10 denoting intolerable pain, and 0 no pain. Ten patients with a total of 24 treatments experienced intolerable pain necessitating premature disruption of the treatment. In 21 of these discontinued treatments involving 9 patients, the photosensitizer used was ALA; only 1 patient treated with MAL-PDT in 3 different anatomical regions requested premature discontinuation. Regions on the head, and especially the cheeks, forehead and scalp proved to be the most painful areas, but the extremities were also sensitive. The differences of PDT associated pain in different anatomical regions, however, were not significant.

The levels of pain in the regions of the head, trunk and extremities during PDT were compared between the groups receiving the different photosensitizers, ALA and MAL. In the head region, MAL-PDT caused significantly less pain than did ALA-PDT. There was a tendency for ALA-PDT to be more painful in all examined anatomical regions, but in the regions of the trunk and the extremities the differences were not significant. The level of pain during the PDT of AK was significantly greater than that in the case of BCC. Despite the difference being significant for the overall AK and BCC groups, breakdown in regard to the two photosensitizers resulted in a significant difference only in the MAL group. In the BCC

and AK groups, significant differences were detected between MAL and ALA in concordance with the observation that MAL-PDT caused less pain.

There was no significant difference in the degree of pain between the genders. Increasing age was significantly associated with more pain sensation. We assessed and compared pain in the following age groups: 40-59 years (n=10), 60-79 years (n=60), over 80 years (n=17), and found significant difference between 40-59 and over 80 years group. A significant difference was not observed between the different methods of pain relief: cooling the skin with wet gauze during treatment, oral analgesia, or both.

4.2. Clinical examination of the efficiency of PDT

4.2.1 Efficiency data throughout a four-year treatment period

In the ALA-PDT group, complete remission was attained in 62,7% in BCC and 57,5% in AK, and incomplete remission in 17,6% in BBC and 23,4% in AK, while there was no response in 19,6% in BCC and 19,2% in AK. For MAL-PDT, the level of complete remission was 67,4% in BCC, and 60,6% in AK, while that of incomplete remission was 19,6 % in BCC, and 27,3 % in AK, and there was no response in 13 % in BCC, and in 12,1% in AK. A second treatment was required for 71 tumors, but because of non-compliance, treatment could only be performed in 63 cases. Cases where there was no response or there was incomplete remission after the second PDT session were followed by surgical excision (29 tumors) or a third PDT session (10 tumors).

4.2.2. In vivo confocal reflectance microscopy with three different wavelength lasers before and after photosensitization and immediately after photodynamic therapy

For routine RCM testing, the 785-nm laser is used most widely. Since two other laser wavelengths (658 nm, 488 nm) were also available for us we first examinated whether there was a difference between the images captured by the three different lasers and whether ALA causes any change on the RCM images. Of one selected AK, images were taken with all three lasers before applying ALA-containing cream, after removing the cream and immediately after illumination. The three different laser images showed no significant difference. The deepest study of the dermis could be done with the 785 nm laser, hereafter we worked with this laser. None of the lasers showed reflectivity associated with the applied ALA.

4.2.3 Reflectance confocal microscopy before photodynamic therapy

In the further phase of the study 12 patients were enrolled, with each patient we chose and treated one selected epithelial tumor according to the study. The PDT-treated lesions were distributed as follows: 4 actinic keratoses, 7 superficial basal cell carcinomas, 1 Bowen's disease. We made RCM examination prior to treatment. In 7 cases a biopsy were also made also prior to this study. All of the lesions showed typical signs appropriate for the three above mentioned diagnoses with RCM and histopathological examination correlated well with the RCM finding.

4.2.4. Fluorescence diagnosis after incubation with the photosensitizer before illumination

After a 3-hour incubation with the photosensitizer, in all cases the fluorescence in UV light was shown clearly in the area of the tumor due to the accumulation of PPIX.

4.2.5 Examination of the immediate effects of PDT with RCM after illumination

Immediately after illumination with RCM edema and vasodilatation in the area of the stratum granulosum and spinosum showed, contours of the epithelial cells became more explicit, and a few inflammatory cells appeared.

4.2.6. RCM at the follow up visit, four weeks after photodynamic therapy

The efficacy of one time PDT was evaluated 4 weeks after treatment, 12 patients were included into the study. One patient with actinic keratosis did not recur for follow-up. In the remaining 11 patients we observed complete remission (CR) in 8 cases (72.7%), incomplete remission in two cases (18.2%) and treatment failure in one patient. Breaking down for diagnosis the efficiency results were as follows: 100% (3/3) complete remission for three AK, one (1/1) partial remission for BD, and 71.4% (5/7) complete remission for sBCCs. One (1/7) sBCC showed incomplete remission and one (1/7) did not respond to treatment at all.

4.2.7. Fluorescence diagnosis for the assessment of treatment efficiency

At 4-weeks follow-up a photos were taken and confocal microscope imaging was performed. In one case of actinic keratosis, ALA was applied once again on the previously treated area for 3 hours. After photosensitization, we examined the extent of fluorescence under UV light. In this case of complete remission, a minimal, diffuse fluorescence was detected, which was even less explicit on the treated area than on the surrounding skin.

In one case of Bowen's disease and one case of superficial BCC we found at follow-up clinically and with RCM incomplete remission and decided to perform PDT a second time. In these two cases a well-defined fluorescence was detectable in the lesions after ALA incubation.

5. DISCUSSION

5.1. Advantages of PDT

When treating epithelial skin tumors, in the choice of therapeutic treatment the primary consideration is efficiency. However, we should bear in mind the cosmetic outcome and the expectations of the patient towards treatment as well. The greatest advantages of PDT are that it is non-invasive, it can be repeated unrestrictedly and it results in an excellent cosmetic outcome. Its proven efficiency rivals surgical treatment, and its cosmetic result is much more favorable. The especially good cosmetic result is due to the fact that the fibroblasts do not accumulate the photosensitizer substance, thus eliminating treatment associated scarring. In contrast to surgical treatment, PDT allows treatment of those precancerous cells, not yet visible to the naked eye, but with an already changed DNA. PDT (as opposed to radiation therapy) does not effect the possibility of subsequent surgery.

5.2. PDT as an evidence based treatment method

By now an international consensus has been born about the treatment with PDT, detailed treatment protocols have been made with the effect that photodynamic therapy is a modern, practical, evidence-based approach for the prevention and treatment of epithelial tumors (actinic keratosis, superficial and nodular basal cell carcinoma, Bowen disease). PDT has proved to be effective in AK, with excellent cosmetic results, the evidence level of this is AI (there is a good evidence to support the use of the procedure obtained of at least one, properly designed, randomized controlled trial). It is also of an AI-evidence-level that for Bowen disease PDT is at least as effective as cryotherapy or 5-fluorouracil, PDT, however it has less side-effects. In the case of sBCC, PDT has proven to be effective, in addition to an excellent cosmetic result with the other advantage of the simplicity of the treatment of multiplex or extensive lesions (AI-evidence level).

5.3. Pain during PDT, the main limiting factor

Intense pain during PDT sessions is the most important undesired reaction, often causing premature termination of treatment, and thus, limited efficiency. PDT associated pain is influenced by several intrinsic (patient-related) and extrinsic (treatment-related) factors. The anatomical region, the diagnosis and the size of the lesion, as well as the degree of photoageing in and around the treatment area are significant intrinsic determinants of treatment related pain, whereas the patient's skin type seems to be unimportant. Lesions localized on the scalp or forehead are more sensitive, compared with the trunk and the extremities. Patients with AK seem to experience more intense pain than those with BCC (possibly due to more advanced photoageing) and, in general, pain increases with lesion size. Age and gender are two intrinsic factors potentially influencing PDT related pain which have been poorly investigated. In some trials gender and age did not influence pain but controversial data was also found. In our study increasing age was significantly associated with more pain sensation.

Extrinsic PDT factors, such as the use of analgesics, type of photosensitizer, light source, wavelength and dose, – at least theoretically – provide the possibility to influence treatment related pain. Several studies have reported attempts to moderate PDT-induced pain, but comparative investigations have not yet been performed. Local anaesthetics have all proven to be ineffective. Cooling with a wet gauze, thermal water spraying, and 1 week pre-treatment with capsaicin (0,075%) have also been tried, but with either no or limited effect. Cold air analgesia and subcutaneous local infiltrative anaesthesia, also proved valuable. Nerve blocks provided effective pain relief during topical PDT for AK on the scalp, forehead or face, and were superior to cold air analgesia.

MAL and ALA are the most widely used topical photosensitizers in the treatment of non-melanoma skin cancer. However, only relatively limited data is available concerning treatment-associated pain using the different photosensitizers. Kasche et al. found that MAL-PDT caused significantly less pain than did ALA-PDT in patients with multiple AKs on the scalp. The reason is probably the greater tumor selectivity of MAL or the fact that ALA, but not MAL, is actively transported to the peripheral nerve endings, triggering nerve stimulation during its excitation. Steinbauer et al. also considered the use of ALA, in contrast with MAL, as a factor predictive of higher PDT associated pain.

In the present study, we evaluated treatment-associated pain during PDT of non-melanoma skin cancer (AK, BCC and BD) in different anatomical regions, using the two photosensitizers, MAL and ALA. We found that in the sensitive head region MAL-PDT was

more tolerable and caused significantly less pain than ALA-PDT. There was a tendency for ALA-PDT to be more painful in all anatomical regions, but in the case of the trunk and the extremities the differences were not significant. PDT of actinic keratosis was significantly more painful than treatment of basal cell carcinomas. Our findings are in accordance with previous studies in regards to the connection between diagnosis and therapy-induced pain. Moreover, the observed significant difference between MAL- and ALA-PDT associated pain in the BCC and AK groups, is in line with the previous observation that MAL-PDT causes less pain. In the case of nine out of ten patients, whose treatment was prematurely terminated due to intolerable pain, the photosensitizer used was ALA. No difference was detected between the methods of pain relief used: cooling the skin with wet gauze during treatment, oral analgesia (paracetamol) before treatment, or both.

Adequate pain relief during PDT presents a difficulty. Our data confirms that while ALA- and MAL-PDT are both equally highly effective for the treatment of non-melanoma skin cancer, MAL-PDT is better tolerated. The lower level of treatment associated pain suggests better suitability of MAL-PDT for the treatment of sensitive anatomical regions or for patients at risk of more pain (e.g. larger lesions or diagnosis of AK, photoageing or field cancerization).

5.4. Adjuvant methods of monitoring the efficiency of PDT

Monitoring the level of PPIX accumulation became possible with the help of fluorescence imaging systems. Photodynamic diagnosis – a method previously used only for verifying the effectiveness of photodynamic therapy – has recently reached a more independent, more important role, as PDD became more quantifiable**.** It has been proved that fluorescence imaging produced highly sensitive and specific results compared with histological examination. It seems that fluorescence diagnosis will be very much suited for early, cost-effective and reliable monitoring and simplifying Mohs surgery. This modified microsurgical method is the most effective method for the removal of high-risk basal cell carcinomas on the face and ears. The procedure of Mohs surgery, however, does involve very high costs, is time consuming and requires the presence of specially trained personnel. Before surgery a Mohs surgeon - in possession of a fluorescence image - is able to plan lines of excision not merely on empirical basis (eye and palpation) but with almost absolute certainty.

In the literature the 785-nm laser is used most commonly for routine RCM examination. Our confocal microscope has three different lasers (488 nm, 658 nm, 785 nm) which we decided to compare. Images were taken with all three available lasers of one

selected actinic keratosis before applying ALA-cream, immediately after removing the cream and finally immediately after illumination. The three different laser images showed no significant difference. The deepest examination of the dermis was made using the 785 nm laser. Based on this experience we continued our worked with the 785 nm laser. We did not see any reflectivity associated with ALA-cream using any of the three lasers. So confocal microscopy with these three lasers seems to be not suitable for the detection of PPIX accumulation in the skin.

Only those cases were regarded as complete remission when the above mentioned deviations and RCM characteristics of epithelial tumors had disappeared and only a residual solar elastosis was observed. Our efficacy data of one-time PDT, measured clinically, and using confocal microscopy, were identical to previously reported data.

At follow-up the most accepted method to judge the effectiveness of treatment and possible recurrence was physical examination (visual, both dermoscopic and tactile). Photodynamic fluorescence, enabling to determine the actual size of the tumor, facilitates to get a more accurate assessment of therapeutic response and possible recurrence. If complete remission cannot be precisely judged at follow-up, a 3-hour MAL or ALA photosensitizer incubation and a fluorescence test under UV light may be performed. If a well-defined area with stronger fluorescence than the surrounding area is detected PDT may be repeated.

Only one publication reported using reflectance confocal microscopic examination for the assessment of therapeutic response subsequent to photodynamic therapy of skin tumors**.** They examined the response of basal cell carcinomas to PDT in patients who suffered from Gorlin's syndrome or from Xeroderma pigmentosum. In these genodermatoses non-invasive diagnosis and treatment methods may greatly improve patient's quality of life. Without PDD and PDT these patients would undergo a number of biopsies and surgical procedures.

To conclude, in the present study the applicability of two non-invasive diagnostic methods was examined before and after photodynamic therapy. In vivo confocal microscopy can give an even more precise diagnosis and is useful for patient follow-up**.** For the extremely precise determination of the edges of a tumor, fluorescence diagnostics is helpful.

Photodynamic therapy is an effective method in treating certain epithelial tumors, it is non-invasive and can be repeated indefinitely with excellent cosmetic results. Each year its dermatological use becomes more and more widespread. Supplemented with fluorescence diagnostics PDT is highly suitable for an effective, even independent treatment of these tumors or for a treatment combined with surgical methods and for a more precise assessment of the effectiveness of the treatment.

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