SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Sun protection and photodynamic therapy as prevention strategies in high skin cancer risk populations

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UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF HEALTH SCIENCES

DEBRECEN, 2019

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The Examination takes place at the 1. seminar room of Department of Preventive Medicine Faculty of Public Health, University of Debrecen at 11:00 AM, October 31, 2019.

Head of the **Defense Committee**:	Prof. Róza Ádány MD, PhD, DSc
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The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen at 13:00 PM, October 31, 2019.

1. Introduction

The incidence of melanoma and non-melanoma skin cancers is increasing worldwide. Certain risk factors, such as fair skin, chronic sun exposure and immunosuppression (eg organ transplantation) increase the risk of the individual developing skin tumors.

1.1. Non-melanoma skin cancers

Basal cell (BCC) and squamous cell carcinoma (SCC) are the most common non-melanoma skin tumors in the Caucasian population. While in immunocompetent fair skinned individuals, BCCs are four times more frequent than SCCs, this ratio is reversed among organ transplants and four times as many SCCs as BCC are expected to occur. Besides UV radiation, ionizing radiation, arsenic exposure, human papilloma virus, immunosuppressive drugs, e.g. azathioprine, cyclosporin A, tacrolimus, and genetic predisposition play a role in their development.

While in BCC intermittent sun exposure, in SCC cumulative UV exposure has a central role in the pathogenesis. Although BCCs almost never metastasize, they are locally invasive, and can also destroy cartilage and bone.

Actinic keratosis (AK) are the precancerous lesions of SCC. They appear as a consequence of chronic sun exposure and are often multiple. Field carcinogenesis is in the background, which means that besides the visible AKs and SCCs, the surrounding keratinocytes may also contain some degree of DNA damage, which can be expected to start malignant process later. In addition to UV-induced DNA damage, UV-induced immunosuppression and inflammation also play a significant role in the pathogenesis of AK and SCC.

1.2. Immune profile of AK and SCC

The role of chronic inflammation in the development of AK and SCC was mainly studied in HPV16 mouse models. The number of human studies is limited in which the immunocomposition of AKs and SCCs has been studied. In these, the immune infiltrate of sun damaged skin and intraepidermal cancer were compared to those in AK and SCC.

In a previous study, the amount of Langerhans cells (LC) decreased during progression to SCC. In human studies, $CD3^+$ T cells were present in the highest number in AK and IEC. The proportion of $CD4^+$ / $CD8^+$ T cells in SCCs was higher, assuming that the $CD4^+$ / $CD8^+$ T cell ratio could be a diagnostic tool for progression towards SCC. In another study, however, the number of $CD8^+$ T cells was higher in SCC and lower in AK.

Overall, elements suggesting immuno-activation and immunosuppression have also been observed in the immune infiltrate of AK in previous studies. The latter promoted progression towards SCC.

1.3 High risk populations

Organ transplanted patients have a higher risk for the development of skin cancer, compared to the immunocompetent population. Primary risk factors are fair skin, high cumulative sun exposure, older age at transplantation, higher and longer immunosuppression. Immunosuppressive drugs used during and after organ transplantation, type of transplantation (heart / lung> kidney> liver) and time since transplantation, the patient's skin type (Fitzpatrick I, skin type II), pre-transplant sun exposure, the presence of p53 gene mutations and lower $CD4^+$ T cell counts contribute to the development of skin tumors.

In addition to SCCs and BCCs, AKs, cornu cutaneum and keratoacanthomas are also common in organ transplanted patients. They are often multiplex and appear predominantly in areas exposed to sunlight, ie in the head and neck region, on the back of the forearms, on the dorsal surface of the forearms, and on the front of the chest in. They often behave more aggressively and give metastases.

Patients with multiple actinic keratosis are generally older, have skin type I. or II and a history of sun exposure during work or free time. AKs appear in the above-mentioned localizations as a result of long-term exposure to UV.

1.4 Prevention strategies

1.4.1. Organ transplanted patients: sun protection, modification of immunosuppression, chemoprevention

The mTOR (mammalian target of rapamycin) inhibitors (sirolimus, everolimus) form a new class of drugs that delay the onset of new skin tumors or hinder the progression of existing tumors. Patients at high risk for developing skin tumors or who have previously had skin tumor removals are advised to switch to a mTOR inhibitor instead of the calcineurin inhibitor. In case of the appearance of multiple NMSCs yearly, systemic retinoids can be introduced as well. In addition, surgical treatment of definitive tumors and field therapies are required to effectively treat field carcinogenesis. However, patients should also be encouraged to use at least 50 factor (SPF50) sunscreen. As part of primary and secondary prevention, it is very important to draw patients' attention to the increased risk of skin cancer, as well as to educate patients on

appropriate sun protection and sunbathing habits and regular self-examination. The number of annual dermatological check-ups should be determined according to the patient's risk factors.
Unfortunately, standardized educational materials are not available for organ transplants. In recent years, many publications have been published on the effectiveness of different educational methods. In these studies, more or less success has been reported in the use of sun protection methods by patients, which, unfortunately, has not been fully improved after training. There is no publication in which patients' sun protection habits were evaluated before transplantation, and then they were also educated.

1.4.2. Treatment of AKs - field therapy

Field therapies such as photodynamic therapy (PDT) can be used to treat chronic sun-damaged skin areas effectively.

1.4.2.1. Photodynamic therapy

PDT induce apoptosis and necrosis of rapidly proliferating precancerous and malignant cells, in addition to destruction of vasculature and activation of innate and acquired immune response and induction of inflammation. It also improves photoaging by indirect effects on dermis. In addition to its therapeutic efficacy, it delivers excellent cosmetic results. Ablative fractional laser pretreatment, presumably through better penetration of the photosensitizer into the epidermis, results in a higher total remission rate.
Due to the progression of AKs towards SCC, immune cell infiltrates associated with lesions change, allowing for the evaluation of prognostic factors such as CD4 / CD8 cell ratio. Animal models have shown that the effect of PDT on the immune cell composition of AKs and SCCs changes. However, very few studies have been conducted to investigate the immunological effects of PDT on healthy human skin and AK. In addition, human AK tissue samples are missing for T cell composition.

2. Aims

1. Epidemiological evaluation of skin cancers of organ transplant recipients cared at the Transplantation Center of University of Debrecen; evaluation of sun bathing and sun protection habits before and after transplantation with a questionnaire and analysis of correlations
2. Comparative clinical, histopathological and immunohistochemical study of conventional PDT (cPDT) and Er:YAG fractional ablative laser PDT (Er:YAG-AFL-PDT) in the treatment of AK and photoaging; analysis of correlations.

3. Patients and methods

3.1. Organ transplanted patients (OTR)

Between January 2016 and July 2016 221 organ transplanted patients participated in the study. All OTRs who were willing to participate in the study were included if at least one summer had elapsed since their transplantation. There were no exclusion criteria. The study received ethical approval from the National Ethical Committee (certificate number: 20989-1/2016/EKU).

3.1.1. Questionnaire and evaluation of skin cancers

Three dermatologists who work at the Department of Dermatology in Debrecen have constructed a questionnaire with 105 questions regarding skin type, date of transplantation, type and number of transplanted organs, immunosuppressive medication history, and education received about increased skin cancer risk during the peri transplantation period. Occupational, recreational and holiday sun exposure before and after transplantation and sun protection methods used before and after transplantation were evaluated. The structure of the questionnaire was mainly based on questionnaires published by Moloney et al., Terhorst et al. and Mihalis et al. All the OTRs who filled the questionnaire, received computer-based education regarding proper sun bathing and sun protection habits, self-examination and skin cancers. These patients received written educational material and sunscreen samples as well.

The calculation of the total sun burden score was based on the factors published by Espana et al.

The MedSol official electronic health record system used at the university served as the source of information about skin cancers.

3.2. Patients with multiple actinic keratosis

Adult (>18 years) patients with severely sun damaged skin and at least fifteen AKs on both forearms, dorsal hands, face or scalp were offered to participate in the study. The trial was approved by the National Ethical Committee (certificate number: 030174/2014/OTIG).

Exclusion criteria included fever, pregnancy, lactation, history of porphyria or other photosensitive disorder (e.g. systemic lupus erythematosus), herpes simplex infection on the treated site, use of medications causing photosensitivity (e.g. retinoids, tetracycline, fluoroquinolones etc.), hypersensitivity to the photosensitizer (5-aminolevulinic acid), prior field treatment, uncontrolled neurological- liver-, heart-, lung disorders, and chemotherapy or immunotherapy in the last 3 months.

3.2.1. Treatment protocol of conventional (cPDT) and Er:YAG ablative fractional laser PDT (Er:YAG-AFL-PDT)

Patient demographic data were recorded, photos were taken and AKs were counted and graded at baseline, 3 month and 12-month control. Treatment area were randomized to receive conventional PDT or Er:YAG-AFL PDT in random manner. One side was pretreated with Er:YAG-AFL (Sciton, ProFractional module, wavelength: 2940 nm; ablation depth: 30-100 μm; density: 22%) immediately before ALA application for laser assisted PDT, while the other side was subjected to conventional ALA PDT. For PDT, 20% of 5-aminolevulinic acid (5-ALA) (5-aminolevulinic acid hydrochloride Biochemica, AppliChem GmbH) was applied under occlusion on both side of the scalp, or face, or dorsum of the forearms and hands after curettage. The area then was irradiated with water-filtered infrared A light (Hydrosun® 501 halogen lamp with 4mm water cuvette at 250 mW/cm2 total irradiance intensity, water filtered spectrum 590-1400 nm) for 20 minutes after 3-hour incubation time. All the patients received one session of treatment without topical anesthesia and all of them wore protective googles during illumination.

3.2.2. Clinical evaluation of photoaging and AK

Patients were evaluated before and 1, 3, 6, 9 and 12 months after treatment by the same investigator. The number and grade of AKs were determined according to Olsen et al, on all visits on both treated sites without knowing the form of treatment. The objective signs of photoaging were evaluated before, 3 and 12 months after therapy. Five-point scale for photodamage - adapted from Dover et al. and Zane et al. - were applied to determine the objective signs of photoaging.

Skin biopsy samples (6mm punch biopsies) of an AK and photographically verified sites of previous AKs were obtained after topical anesthesia (lidocaine hydrochloride) before PDT, 48 hours and 3 months after PDT.

3.2.3. Histology, immunohistochemistry

Skin samples were fixed with 10% formaldehyde, embedded in paraffin and cut into 3 μm-thick sections. The sections were deparaffinized, the endogenous peroxidase activities were inactivated in 3% H2O2 for 15 minutes. Antigen retrieval was achieved by pressure-cooking of tissue samples in Tris-EDTA buffer (pH 9). Sections were blocked with 1% fetal bovine serum (Biosera, Nuaille, France) in Tris-buffered saline (TBS) for 1 hour in room temperature. Sections were stained with antibodies as follows: CD3 (Biocare Medical, Pacheco, CA, USA;

clone EP41 ;1:100), CD4 (Leica Biosystems, Wetzlar, Germany; clone 4B12; prediluted), CD1a (Abcam, Cambridge, UK; clone C1A/711; 1:600), CD8 (Cell Signaling, Danvers, MA, USA; clone C8/144B; 1:1200), p53 (Biocare Medical, Pacheco, CA, USA; clone DO-7; 1:100), Ki67 (Biocare Medical, Pacheco, CA, USA; clone SP6; 1:100). Sections were incubated with the primary antibody overnight at 4 °C, then HRP-conjugated secondary antibody (One-Step Polymer HRP Reagent; BioGenex, CA, USA) was employed for 30 minutes at room temperature. Staining was detected by DAB Chromogen (BioGenex, Fremont, CA, USA), Vector® VIP and ImmPACTTM NovaREDTM Kit (p53 and Ki67 staining, VECTOR Laboratories, Burlingame, CA, USA). Methyl green stain was also performed in p53 sections.
One of the serial sections prepared from the tissue blocks were stained with hematoxylin-eosin for histopathological evaluation.

The number of keratinocytes with positive p53 and Ki67 staining, the number of CD1a+ epidermal Langerhans cells, and dermal CD3+, CD4+ and CD8+ T cells were evaluated in blinded manner by a dermatologist and a pathologist. A score was calculated as the average number of cells in question per high power field (hpf, x100 magnification) counting the positively stained cells in three hpf for each section.

3.2.4. Statistical analysis

The following statistical methods were used: descriptive statistical methods, Chi-squared test, Fisher's exact test, unpaired t-test, multinomial logistic regression, Kolmogorov-Smirnov test, Wilcoxon matched-pairs signed rank test, Friedman and Dunn's post hoc test, and Spearman's rank correlation tests. Statistical significance was established at the 5.0% significance level. If the 95% confidence intervals did not overlap, the differences were considered statistically significant. Odds ratios (OR) was calculated with the corresponding 95% upper and lower CI. The logistic regression models were adjusted for age and sex.

Internal validity was measured by Cronbach's alpha. The different types of questions were analyzed separately. The values for the questions about sun protection methods and sunbathing habits were between 0.61-0.94, confirming a moderate-to-strong internal consistency of the questionnaire.

SPSS (version 19.0, SPSS Inc., Chicago, IL, USA) software was used for the statistical analyses. The data were graphically represented using Microsoft Excel 2010 (version 11.5612.5606; Microsoft, Redmond, WA, USA).

4. Results

4.1. Epidemiological evaluation of skin cancers of organ transplant recipients cared at the Transplantation Center of University of Debrecen and assessment of primary prevention

4.1.1. Patient population

221 kidney transplant patients participated in the study; the mean post-transplantation time was 8.7±7.1 years. The mean age at the time of transplantation was 44.5±14.1 years, and the mean age at the time of the administration of the questionnaire was 52.6±12.9 years. 91% of the patients had received one, 8% 2 and 0.9% 3 kidney transplant. The male to female ratio was 1.66:1. All patients were Caucasian and most had Fitzpatrick skin types II (38.0%) and III (45.7%). Azathioprine was administered to 7.2% of the patients, and 33.9% had previously received CsA treatment. At the time of the questionnaire, the main immunosuppressive regimen was a combined treatment with TAC, MMF and methylprednisolone (43.4%). An average of 56.5 months (4.7 years) had elapsed since the patients' first dermatological examinations. Although 90% of the patients had worked before transplantation (mainly indoors), only 38.9% returned to work after transplantation.

4.1.2. Sun exposure before and after transplantation

Occupational, recreational (after work and over the weekends) and holiday sun exposure before and after transplantation were analyzed in four life periods, and total sun burden scores (TSB) were calculated.

On a work day, significantly more patients were exposed to sunlight between 11 am and 3 pm or after 3 pm, and patients had more than 4 hours of sun exposure a day before transplantation than they had after transplantation. Before the age of 20, 75.7% of patients spent more than 20 hours a week in the sun, while between the ages of 20-40, 64.2% of patients experienced that level of sun exposure. After transplantation, the number of patients who worked significantly decreased, and people who worked mainly held office-based positions rather than participating in work based outside. After transplantation, more patients avoided sun exposure, and most patients spent less than 1 hour a day in the sun, even on a work day.

Before transplantation, significantly more OTRs were exposed to sunlight independent of the time of day and had at least 3 hours of sun exposure a day during weekends. After transplantation, most patients avoided sunlight or were exposed to sunlight before 11am, for a maximum of 1-2 hours a day.

Significantly more patients went on holidays and spent more than 4 hours a day in the sun, mostly independent of the time of day, before transplantation.

Moreover, 58.4% of the patients had high TSB scores before transplantation, but after transplantation, only 10.9% had high TSB scores. The factors influencing high TSB after transplantation were Fitzpatrick skin type I and high TSB before transplantation.

4.1.3. Sun protection habits before and after transplantation

Before transplantation, most of the patients preferred staying in the shade (74.2%) and wearing sunglasses (48.4%), although only 35.7% reported the use of sunscreens. The patients reporting sunscreen use did so almost exclusively in the summer and mainly used SPF 15-30 and SPF 30 products. Only 12.7% of the OTRs reported always applying sunscreen before transplantation. After transplantation, the rate of OTRs who reported not using any sun protection methods marginally decreased, although the preferred sun protection methods remained the same, with a slight increase in staying in the shade (78.7%) and a decrease in sunscreen usage (33.9%). Although the preferred SPF of the sunscreen products was SPF 50, only 16.8% of patients always applied it, almost exclusively in the summer. The differences between pre- and post-transplantation sun protection methods were not statistically significant.

4.1.4. Education about increased skin cancer risk

65.2% of the patients reported receiving verbal counselling by transplantation physicians about their increased skin cancer risk at the time of transplantation. However, these self-reported educated patients did not use better sun protection methods than the non-educated OTRs, and there was also no significant difference in the duration and time of day of their sun exposure after transplantation.

4.1.5. Epidemiology of skin tumors

Among the OTRs who participated in the study, 29 (13.1%) had at least one surgery after transplantation to remove skin cancer, mostly from the head/neck region. 15 patients had multiple skin cancers. Most of the patients had Fitzpatrick II (41.4%) and III (51.7%) skin types. Five skin cancers (4 BCC and 1 basosquamous carcinoma) were removed from 5 patients even before transplantation. The remaining 92 skin cancers were removed after transplantation. Basal cell cancer was the most frequent histological diagnosis. The ratio of basal cell cancer to squamous cell cancer was 1.15:1. An average of 95.5±69.1 months elapsed after transplantation before the appearance of the first skin cancer. Patients with skin cancer were on average older

(p<0.001) (50.8±9.1 years) than those with no skin cancer (43.6±14.4 years), and patients who developed skin cancer had undergone a longer duration of immunosuppression (156.3±71.3 months versus 91.0±83.2 months, p<0.001). Fitzpatrick skin types did not correlate with the patients' histories of skin cancer. Before transplantation, 69% of the patients had high total sun burdens. After transplantation, 10.3% of patients were receiving azathioprine, 58.6% were receiving therapy, and 37.9% of the patients were later administered mTOR inhibitors.

Significantly more patients with skin cancer reported having engaged in recreational sunbathing independent of the time of day (p=0.005), and 41.4% of the patients who developed skin cancer spent more than 4 hours a day in the sun before transplantation. However, there was no statistically significant difference in the duration or time of day of pre- and post-transplantation occupational sun exposure between patients with and without skin cancer.

Among patients with skin cancer, the most preferred sun protection methods before transplantation were seeking shade (72.4%) and using a hat (48.3%), while only 31.0% of the patients applied sunscreen. After transplantation, seeking shade (86.2%) and wearing a hat (44.8%) remained the most frequently reported methods, but more patients reported using sunscreen (41.4%).

4.2. Comparative study of the efficacy of Er:YAG-AFL-PDT and cPDT in the treatment and prevention of AKs and photoaging and on the induced changes in immune profile

4.2.1. Patient population

Eleven patients (average age 77±6,9 years) with a total number of 427 AKs completed the study.

There was no statistically significant difference in the initial number of AKs on the two differently treated sides (p=0.769).

4.2.2. Therapeutic efficacy

The number of AKs has significantly decreased 3 months after both Er:YAG-AFL PDT and cPDT by 87.56±17.30% and 82.56±16.53% (p=0.039). At 12 months follow up Er:YAG-AFL PDT and cPDT showed 69.45±30.94% and 66.9±25.41% (p=0.844) decrease in the number of AKs. Er:YAG AFL pretreatment induced higher therapeutic efficacy in those patients where PDT was highly effective (rs=0.838, p=0.002).

4.2.3. Photorejuvenation effects

Both treatment modalities significantly improved global photoaging, mottled pigmentation, the roughness of the skin and both decreased significantly fine wrinkles 3 months later. There was no difference in the efficacy of the treatments.

4.2.4. Histological changes

Analyzing hematoxylin-eosin stained tissue sections we found prominent dysplasia and solar elastosis, and a moderate inflammatory cell infiltration in AK samples before treatment. 48 hours after Er:YAG-AFL PDT and cPDT, a prominent inflammation with acantholysis and necrosis could be observed. 3 months later, both dysplasia and solar elastosis decreased.

4.2.5. Immunohistochemical evaluation

4.2.5.1. Epidermal changes

p53 expression

The number of $p53^+$ keratinocytes significantly decreased at 48 hours and it was also significantly lower at 3 months after both Er:YAG-AFL-PDT ($p=0.004$ and $p<0.001$) and cPDT ($p<0.001$ and $p<0.001$) compared to the corresponding initial AK field. Significant difference was not seen between the two treatments at the time points ($p=0.559$ and $p=0.651$).

Ki67 expression

The number of $Ki67^+$ cells decreased as well 48 hours and 3 months after both treatments, but it was only statistically significant in case of Er:YAG-AFL-PDT ($p=0.002$ and $p=0.009$).

CD1a+ Langerhans cells

The number of $CD1a^+$ epidermal Langerhans cells significantly decreased 48 hours after both Er:YAG-AFL-PDT ($p<0.001$) and cPDT ($p=0.017$). 3 months later their number returned almost to the initial level in both treatment groups.

4.2.5.2. Dermal changes

We found positive correlation between the number of $CD1a^+$ Langerhans cells and the number of $CD3^+$ dermal T cells in all samples (baseline samples $rs=0.757$, $p=0.009$, samples after 3 months Er:YAG-AFL PDT $rs=0.714$, $p=0.016$; cPDT $rs=0.744$, $p=0.011$). Baseline $CD3^+$ T cell count in AKs correlated with the decrease in p53 staining 3 months after Er:YAG-AFL PDT ($rs=0.683$, $p=0.024$), and initial number of $CD3^+$ T cells also correlated with 3 months therapeutic efficacy of cPDT ($rs=0.731$, $p=0.013$).

Although there was no significant difference in the number of $CD3^+$ and $CD4^+$ T cells 48 hours and 3 months after either Er:YAG-AFL- PDT ($p=0.998$ and $p=0.103$; $p=0.330$ and $p=0.999$) or cPDT ($p=0.999$ and $p=0.872$; $p=0.999$ and $p=0.999$), we observed a decrease in the amount of

$CD4^+$ T cell infiltrate 48 hours after both PDT treatments. $CD4^+$ T cell count reached almost the initial level at 3 months. The number of $CD3^+$ T cells remained almost unchanged 48 hours after both treatments, but 3 months later their number decreased, especially after Er:YAG-AFL PDT. The number of $CD8^+$ T cells also decreased 48 hours after Er:YAG-AFL- PDT ($p=0.274$) and cPDT ($p=0.999$), and their number remained low 3 months later. In fact, the number of $CD8^+$ T cells 3 months after Er:YAG-AFL- PDT was significantly ($p=0.013$) lower than the baseline $CD8^+$ T cell count.

5. Discussion

1. Epidemiological evaluation of skin cancers of organ transplant recipients cared at the Transplantation Center of Debrecen and assessment of primary prevention

Several publications have been published in previous years on the increased skin cancer risk of OTRs. It is also well known that their risk can be reduced by the use of appropriate sun protection methods, including sunscreens. In order to promote the use of these methods of sun protection, several attempts have been made in patients who have already undergone transplantation in recent years through the use of leaflets, workbooks, video recordings, confirmation emails, and mobile applications.

Unfortunately, however, they did not lead to a breakthrough. In addition, there have been no studies in the past that reported the habits and education of the patients before the transplantation. However, perhaps with the education of this group of patients, greater compliance and reduced exposure to sunlight and more effective use of sun protection methods could be achieved.

In our study, we have also investigated the pre-transplant sunbathing and sun protection habits uniquely among transplanted patients. These patients received a computerized presentation on increased skin tumor risk, proper sunbathing and sun protection habits, self-examination, and received written educational leaflets and sunscreen samples as well. Pre-transplant total sun burden (TSB) values were high in the majority of patients, and these patients were also more likely to have high post-transplant TSB. Although besides the decrease in post-transplant TSB, working, recreational and holiday sun exposures also decreased, which is more likely to be due to socioeconomic and lifestyle changes. Only 33.9% of patients used sunscreen, and only 16.8% of them used it regularly.

65.2% of patients replied that they had been informed about the increased risk of skin cancer, but had not applied better sun protection methods and there was no difference in sun exposure compared to patients who had not been informed.

We found a correlation between pre-transplant recreational/ weekend sun exposure and post-transplant skin cancer. Higher percentage of skin cancer patients (41.4%) applied sunscreen. These patients were older and had longer duration of immunosuppression. The ratio of BCC to SCC was 1.15: 1, which is different from that in larger studies.

On average, 4.7 years have passed between transplantation and the first dermatological examination of patients. This data has also confirmed the findings of two previous studies of the need for closer cooperation between transplantation physicians and dermatologists.

The evaluation of pre-transplant sun-safe behavior and TSB highlighted the need for earlier intervention programs, focusing more on sunscreen use and recreational sun exposure and targeting not only OTRs but also patients who are likely to become OTRs in the future.

2. Comparative study on the efficacy of Er:YAG-AFL-PDT and cPDT in the treatment and prevention of AKs and photoaging and in the induced changes in immune profile

In case of chronic sun damaged skin and multiple AKs, field therapy is needed for the effective treatment of these lesions. PDT is one of a field therapies, which has excellent cosmetic outcome, however the reported therapeutic efficacy is within a wider range (59-92%). Furthermore, one treatment is not enough, due to recurrences after 1 year.

In the current study, 3 months after one PDT treatment, the number of AKs decreased significantly and the therapeutic efficacy was 82.56%. Although, 12 months later, new AKs appeared on the previously treated sites and the efficacy decreased to 66.9%. Immunohistochemical staining supported the cause of the decrease in efficacy: although the number of $p53^+$ and $Ki67^+$ keratinocytes decreased 3 months after treatment; they were not eliminated totally. It gave opportunity for the appearance of new AKs.

In accordance with previous publications, fractional ablative laser pretreatment increased the efficacy of PDT. The therapeutic efficacy was 87.56% and the number of newly developing AKs was less as well.

Regarding photoaging, there was no significant difference between the 2 treatment modalities, both improved global photoaging, hyperpigmentation, roughness of the skin and the number of fine wrinkles also decreased 3 month later.

The number of publications about the immune infiltrate of AK and SCC is increasing. However, only few studies analyzed the effect of PDT on immune infiltrate of AKs.

The available data are from mouse SCC models and healthy human skin examinations. In these studies, the number of $CD4^+$ and $CD8^+$ T cells increased 1 week and 4 hours after PDT; moreover, not consequently in one, the number of $CD1a^+$ Langerhans cells decreased, while in the other their number increased 24 hours after the treatment on the treated site.

In our study, we found changes in the immunocomposition of AKs.48 hours after Er:YAG-AFL- and cPDT, the number of $CD1a^+$ Langerhans cells, $CD4^+$ and $CD8^+$ T cells decreased, although the average $CD4^+/CD8^+$ cell ratio remained unchanged.

Other changes could be observed 3 months later. The number of $CD1a^+$ Langerhans cells almost returned to the baseline level and the average $CD4^+/CD8^+$ T cell ratio increased due to the further decrease of the number of $CD8^+$ T cells.

Probably the $CD8^+$ T cells are responsible for the induction of immune response against dysplastic keratinocytes. Although we could not find correlation between the efficacy of PDT and the initial number of $CD8^+$ T cell count. It seems that if there are more $CD1a^+$ Langerhans cells and $CD3^+$ T cells in the AKs, the efficiency of PDT is higher. Such a study has not been conducted for human AK before.

6. Summary

The amount of sun exposure before transplantation was shown to be important for post-transplant skin cancer risk, underlining the pathogenetic role of chronic sun damage and juvenile high UV exposure in the development of skin cancer. Although sun exposure after transplantation was lower, knowledge of effective sun protection and its practical application remained incomplete.

In the current population, the proportion of basal cell carcinoma was higher than that of squamous cell carcinoma.

In addition, only 65.2% of the patients remembered being informed about the increased risk of skin cancer, but they did not use more effective sunbathing methods and sun protection habits, and dermatological examination was conducted only several years after transplantation. These results also confirmed the need for closer cooperation between professions, and that patients with chronic kidney disease who are candidate for transplantation should be targeted towards educational lectures and dermatological examination.

The therapeutic results achieved among patients with multiple AK confirmed that AKs can be effectively treated and even their development can be reduced with PDT. However, one treatment is not enough to completely eliminate atypical keratinocytes. Er: YAG fractional ablative laser pretreatment can increase the effectiveness of PDT, but in this case, the

appearance of new AKs should be considered later as well. Based on our results, repetition of PDT treatment is necessary to permanently eliminate AKs. In both forms, PDT has been shown to be effective in improving the symptoms of photoaging. Based on our results in the study of AK immune infiltrates, it seems to be a legitimate assumption that the immune cell composition of AKs affects the efficacy of PDT, or indirectly, to indicate the immunogenicity of AK-infiltrating cells.

Thus, sun protection and photodynamic therapy are two promising prevention strategies in populations with high skin cancer risk, where there is still opportunity for improvement.

7. New statements

1. Pre-transplant TSB values were high in the majority of patients, and these patients were also more likely to have a high TSB after transplantation.
2. Although only 10.9% of patients had a high TSB after transplantation, there was a significant reduction in the number of patients who worked and went on vacation.
3. Only 1/3 of the patients used sunscreen and within that only 16.8% used it regularly.
4. Educated patients did not apply better sun protection methods and there was no significant difference in the amount of sun exposure compared to the non-educated patients.
5. Recreational sun exposure before transplantation was a significant risk factor among patients with skin cancer.
6. Older age and longer duration of immunosuppression were in connection with the appearance of skin tumors.
7. 13.1% of the kidney transplanted patients had at least one skin cancer removal and the ration of BCC: SCC was 1.15:1.
8. Intervention programs targeting patients before transplantation are needed with an even greater emphasis on reducing sun exposure and applying sunscreen.
9. Efficacy of PDT can be increased with Er:YAG ablative fractional laser pretreatment. However, there was no difference in the improvement of the signs of photoaging.
10. The number of $p53^+$ and $Ki67^+$ keratinocytes decreased 3 months after both treatments, but they were not completely eliminated, creating a chance for newer AKs.
11. The immune infiltrate of AKs changed 48 hours and 3 months after both treatments.
12. If the number of $CD1a^+$ Langerhans cells and $CD3^+$ T cells are higher in AKs, the efficacy of PDT is also higher.

8. Key words

photoprotection, photodynamic therapy, organ transplantation, actinic keratosis, non-melanoma skin cancer, prevention

9. Acknowledgments

I would like to express my grateful thanks and appreciation to my supervisor, Dr. Gabriella Emri, who has always supported my ideas and helped me to realize them with her valuable professional knowledge.

I am grateful to Prof. Dr. Éva Remenyik, who helped me as my second supervisor during my work and also supported me with her professional skills throughout my work.

I am grateful for the great help to Eszter Janka in the statistical calculations, to Eszter Fidrus in the lab work, to Tibor Gáll in the digitization of the questionnaires, and to Sándor Kollár for the histological discussions.

I would like to thank Dr. Balázs Nemes for making it possible to organize the dermatological care of kidney transplant patients and to develop cooperation.

In addition, I am grateful to Dr. Bence Gábor Papp, who, as a medical student, has undertaken a huge amount of work in the questionnaire survey of transplanted patients.

I would like to thank József Oláh for taking photos and cheerful mood.

I would like to thank my husband, Gergő and my parents, who have always supported me to reach my goals.

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Subject: PhD Publikációs Lista

Candidate: Emese Gellén
Neptun ID: X5E874
Doctoral School: Doctoral School of Health Sciences

List of publications related to the dissertation

1. **Gellén, E.**, Fidrus, E., Janka, E., Kollár, S., Paragh, G. J., Emri, G., Remenyik, É.: 5-Aminolevulinic acid photodynamic therapy with and without Er:YAG laser for actinic keratosis: changes in immune infiltration.
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Bőrgyógyász. Venerol. Szle. 90 (1), 30-36, 2014.

Total IF of journals (all publications): 24,511
Total IF of journals (publications related to the dissertation): 7,728

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

16 May, 2019

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