Preclinical evaluation of diagnosis of actinic keratosis and cutaneous squamous cell carcinoma

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The objective of the present study is to investigate the possbility to discriminate between actinic keratoses an dcutaneous squamous cell carcinoma with fluorescence diagnosis.

Ethical review	Approved WMO
Status	Recruiting
Health condition type	Skin neoplasms malignant and unspecified
Study type	Observational invasive

Summary

ID

NL-OMON32657

Source
ToetsingOnline

Brief title
FDAP in actinic keratosis and cutaneous squamous cell carcinoma

Condition

- Skin neoplasms malignant and unspecified

Synonym
solar keratosis

Research involving
Human

Sponsors and support

Primary sponsor: Universitair Medisch Centrum Sint Radboud
Source(s) of monetary or material Support: financiering die gegenereerd is uit eerder klinisch trial onderzoek wordt hiervoor gebruikt

Intervention

Keyword: actinic keratosis, fluorescence diagnosis, squamous cell carcinoma

Outcome measures

Primary outcome

The difference in fluorescence intensity between actinic keratoses and squamous

cel carcinoma

Secondary outcome

Thickness of the stratum corneum in the different diseases

Study description

Background summary

The potential value of FDAP as a non-invasive diagnostic procedure to discriminate between different stages of keratinocytic intraepidermal neoplasias is described in paragraph 4.2. In this study, FDAP was performed in patients with field-cancerization after keratolytic pre-treatment. Subsequently biopsies from different lesions were taken. (Immuno)histochemistry was performed on these biopsies for histopathological classification, using the KIN- as well as conventional classification, and assessment of Ki67-antigen expression and stratum corneum thickness.
Although KIN III lesions tended to have increased lesional:non-lesional fluorescence ratios compared with KIN I and KIN II lesions, no statistically significant differences between the different KIN lesions could be demonstrated. Six lesions classified as verrucous hyperkeratoses had significantly lower fluorescence ratios (mostly around 1) compared with normal skin, KIN I, KIN III and miSCC. The three SCCs that were biopsied had significantly higher fluorescence ratios compared with KIN II lesions and the verrucous hyperkeratoses. Analysis of proliferative activity as assessed by immunoreactivity for the Ki67 antigen also did not reveal any significant differences in fluorescence ratios among the three levels of Ki67 expression, which is not surprising since Ki67 expression and KIN grade are highly correlated. However, when the lesions were divided into four diagnostic categories (true/false positives/negatives), with a fluorescence ratio of 1.0 taken as a cut-off value and KIN lesions/SCC and verrucous hyperkeratoses/normal skin classified as (pre)malignant and benign lesions, respectively, lesions with a fluorescence intensity lower than surrounding skin

(fluorescence ratio < 1.0; true and false negatives) were found to have a significantly lower number of Ki67+ cells than lesions with a fluorescence ratio greater than 1.0 (true and false positives). Additionally, when Ki67 immunoreactivity from all four diagnostic categories was mutually compared, a significant difference between the false- and true-positive lesions and between the true-positive and -negative lesions was found. However, the reason for this would probably be the relative high number of true positive lesions which were mostly all hyperproliferative lesions. So, proliferative activity seems to be a confounder rather than the cause of high fluorescence.
When macroscopic fluorescence values were plotted against the stratum corneum thickness, a negative correlation was found indicating hyperkeratosis to be an important hindering factor with respect to PpIX accumulation. Analogously, lesions with a fluorescence intensity lower than surrounding skin (fluorescence ratio < 1.0; true and false negatives) had a significantly thicker stratum corneum than lesions with a fluorescence ratio greater than 1.0 (true and false positives). Moreover, in the true- and false-positive lesions the mean stratum corneum thickness was found to be significantly lower compared with the false-negative lesions. A summary of these data can be found in table II in paragraph 4.2.
From the above it was concluded that FDAP cannot be used to discriminate between various KIN-stages. However a tendency towards higher macroscopic fluorescence was observed in KIN III lesions compared with KIN I/II lesions. This may be related to an increased number of epidermal cells in KIN III lesions (epidermal hyperplasia). Although there was a tendency towards an increased stratum corneum thickness in KIN III lesions compared with KIN I/II lesions, no statistically significant differences were found. It may be imagineable that a slight thicker stratum corneum in KIN III lesions could decrease the fluorescence contrast between the KIN I/II and KIN III lesions. Furthermore, macroscopic fluorescence seems independent of proliferative status but it is negatively correlated with stratum corneum thickness. Despite the fact that the patients in our study received a keratolytic pre-treatment with salicylic acid in petrolatum for 1-2 weeks prior to FDAP, hyperkeratosis still seemed to be responsible for variations in macroscopic fluorescence. As penetration of the hydrophilic ALA through the lipophilic stratum corneum is a prerequisite for PpIX formation, adequate keratolytic pre-treatment is essential for optimal results with PDT and FDAP.
Considering the high fluorescence observed in SCC, it might be interesting to perform further studies whether FDAP can be useful to discriminate between KIN and invasive carcinoma.

Study objective

The objective of the present study is to investigate the possbility to discriminate between actinic keratoses an dcutaneous squamous cell carcinoma with fluorescence diagnosis.

Study design

Fluorescence diagnosis and biopsy procedure
Prior to the day FDAP takes place, the skin areas under study will be pretreated with 5 or 10% salicylic acid in petrolatum for 1 week, depending on the clinical picture, to get rid of excess scales and hyperkeratosis until clinically satisfactory results were achieved. On the day of the FDAP procedure 20% ALA-cream (Medac Gmbh, Wedel; Germany) will be applied under an occlusive dressing to these skin areas. After 3 hours of incubation fluorescence intensity on the skin will be recorded using a digital fluorescence imaging system (DyaDerm, Biocam GmbH, Regensburg; Germany). This system consists of a flash light (Xenon light source with a custom band pass filter (370-440 nm)) and a 12-bit Sony CCD camera combined in one adjustable arm coupled to a Pentium IV computer equipped with custom made image capturing software (Dyaderm Pro v1.4, Biocam GmbH, Regensburg; Germany). The flash light emits 7 light pulses per second to the skin which are recorded by the CCD camera (exposure time 100 *sec) equipped with a special Schott GG 455 long pass filter to filter out the excitation light. As PpIX fluorescence emission consists of light in the red spectrum, the red pixels of the CCD camera were used to generate a fluorescence image. In this way a normal coloured image and a fluorescence image were processed in real-time. Because of the short exposition time to the excitation light, photobleaching of PpIX was minimized in this way. To correct for different lighting environments between pictures, a fluorescence reference standard (Maccal 8044, 738-00, Multifoil, Utrecht; The Netherlands) was included on every image. Images were recorded in 16-bit greyscale TIFF format. After FDAP, 4 mm punch biopsies will be taken from selected lesions/skin areas of interest under local anesthesia with 1% xylocain-adrenalin.

Analysis of fluorescence images
16-bit greyscale TIFF fluorescence images will be imported in NIH ImageJ software (http://rsb.info.nih.gov/ij/). Because the Xenon light source used for excitation had the highest intensity in the center of the illuminated area, shading correction was performed by means of the following algorithm:
with
B = blank image (image from a white homogeneous background recorded with the Dyaderm system), BImax = highest intensity of blank image, I = (uncorrected) image, C = normalised shading image, S = shading corrected image.

Histopathology and immunohistochemistry
6 µm slices will be fixed on a glass slide, deparaffinized, hydrated and washed in PBS subsequently. For Ki67-antigen staining the sections will be first pretreated in citrate buffer (pH 6.0) using the microwave antigen-retrieval method. Afterwards, immunohistochemical analysis will be performed after blocking for endogenous peroxidase using the Powervision (Immunologic, Duiven, The Netherlands) staining system with diaminobenzidine (DAB) as chromogen. The sections will be incubated for one hour with the primary MIB-1 antibody (Dako,

Heverlee, Belgium; 1:100) directed against the cell-cycle associated antigen Ki67. For counterstaining Mayer*s haematoxylin will be used.
Also haematoxylin-eosin staining will be performed on every slide for assessment of the histopathological diagnosis and KIN-classification. All slides will be assessed by one and the same pathologist (WB) for uniformity.

Immunohistochemical and digital image analysis
Immunoreactivity for the Ki67-antigen will be scored semi-quantitatively in the following manner according to Keating et al.13: 0 = only basal layer positivity, 1 = positivity confined to basal 1/3 of the epidermis, 2 = positivity confined to basal 2/3 of the epidermis, 3 = transepidermal positive staining.
Analysis of digital microscopic images will be done using ImageJ digital image analysis software. Digital photographs of the HE-sections were made at 50x magnification. To determine the thickness of the stratum corneum the average thickness will be calculated as total stratum corneum surface including hyperkeratosis per millimeter length of the stratum corneum in different biopsy sections.

Statistical analysis

To analyze Ki67-expression and KIN-grade in relation to fluorescence intensity one-way analysis of variance (ANOVA) will be used. For analysis between groups Duncan*s post-hoc test will be performed. Analysis of fluorescence intensity in AK and BD, Ki67-expression and stratum corneum thickness between the 4 diagnostic categories will be performed using an unpaired Student*s t-test. Correlation analysis of macroscopic fluorescence and stratum corneum thickness will be performed using Pearson*s R. All statistical calculations will be performed using Statistica 6.0 s software (Statsoft Inc., http://www.statsoft.com) and Microsoft Excel 2000. A p-value < 0.05 will be considered statistically significant.

Study burden and risks

The patients will be in hospital during 5 hours. At first the cream will be applied and the patients will have to wait during 3 hours. Afterwards the cream will be removed and the fluorescence intensity will be measured as described above. From a maximum of four lesions 4 mm biopsies will be taken. Part of this procedure is standard. Only the application of the cream, the waiting and measurements of the fluorescence intensity is extra.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

Patients with actinic keratoses as well as squamous cell carcinoma
Age above 18 years

Exclusion criteria

Pregnancy or breast feeding
History with photodermatosis
Systemic or topical treatment interfering with the disease

Study design

Design

Study type: Observational invasive

Masking:	Open (masking not used)
Control:	Uncontrolled
Primary purpose:	Diagnostic

Recruitment

NL

Recruitment status:	Recruiting
Start date (anticipated):	03-09-2009
Enrollment:	20
Type:	Actual

Ethics review

Approved WMO

Date:	03-03-2009
Application type:	First submission
Review commission:	CMO regio Arnhem-Nijmegen (Nijmegen)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL26106.091.08