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Calcipotriol as a daylight photodynamic therapy enhancer: a case-control study

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Abstract
Actinic keratoses (AKs) are common skin lesions found on sun-exposed areas and are considered potential precursors to squamous cell carcinomas (SCCs). This observational case-control study evaluates the efficacy of combining traditional daylight photodynamic therapy (DL-PDT) with pre-treatment using 0.005% calcipotriol (CAL) ointment. Twenty immunocompetent male patients with grade I-II AKs on the scalp and/or face were randomized into two groups: the case group received a 14-day pretreatment with CAL ointment before DL-PDT, while the control group used a moisturizing cream. Both groups underwent a series of three DL-PDT sessions. The study utilized the Actinic Keratosis Area Severity Index (AKASI) scoring system to measure the actinic damage at baseline, 3 months, and 6 months post-treatment. Results showed a significant reduction in AKASI scores in the CAL group compared to controls, indicating that CAL pretreatment enhances the efficacy of DL-PDT. This combination treatment was well tolerated, with minimal discomfort reported. The findings suggest that incorporating CAL into the treatment regimen can improve the clearance of AKs and potentially prevent their progression to SCCs.

Introduction
Actinic keratoses (AKs) are a common skin disease that usually arises on photodamaged skin. More than 75% of AKs develop on sun-exposed areas such as face, scalp, neck, forearms, and dorsal hands. The term actinic cheilitis refers to AKs occurring on the mucosal surface of the lips.[1] The presence of numerous subclinical and clinically apparent lesions is termed field cancerization, a condition that heightens the risk of developing non-melanoma skin cancer (NMSC).
AKs are considered potential precancerous lesions; indeed, 60-65% of squamous cell carcinomas (SCCs) originate from pre-existing AKs.[2] Consequently, patients with multiple AKs face an increased cumulative risk of progressing to SCC.[3]
Numerous therapeutic options exist for AKs, ranging from treatments for individual lesions, such as cryotherapy and CO_2 laser therapy, to field treatments (such as home-based creams containing 5-fluorouracil [5-FU], imiquimod or tirbanibulin).[4,5] A widely used treatment is photodynamic therapy (PDT), which utilizes a cream containing either 5-aminolevulinic acid (ALA) or 5-methyl-aminolevulinic acid (MAL).[6] These agents induce the accumulation of protoporphyrin IX, a photosensitizing agent, in abnormal keratinocytes. Exposure to sunlight (daylight PDT- DL PDT) or red light from an LED lamp (conventional PDT – C-PDT) induces the accumulation of oxygen radicals (ROS), leading to in necrosis and apoptosis of precancerous and cancerous cells present in the treated field of cancerization.[7] PDT is highly effective and well tolerated by patients, particularly DL-PDT, which requires less time and is less painful.[8,9]
Calcipotriol, a vitamin D analogue approved for the treatment of psoriasis, is a recently introduced molecule in the landscape of treatment for AKs. It increases the production of protoporphyrin IX, promotes keratinocyte differentiation, and induces the expression of thymic stromal lymphopoietin (TSLP) in keratinocytes. TSLP represents a significant inducer of antitumor immunity in the cutaneous defensive barrier. This has the potential to significantly enhance efficacy in the treatment of skin tumors, eliminating existing lesions and preventing the onset of new ones.[10]
Some studies have observed the use of calcipotriol in combination with 5% 5-FU and conventional PDT, indeed demonstrating an enhancing effect in the clearance of actinic keratoses.[11,12]

Materials and Methods
Twenty patients with actinic keratoses on the scalp only or scalp and face attending the photodynamic therapy outpatient clinic presented were selected for the observational case-control study.
Inclusion criteria comprised immunocompetent patients with grade I-II AKs on the scalp or both the scalp and face who accepted to sign the necessary informed consent. Conversely, exclusion criteria encompassed immunocompromised individuals, including those with autoimmune diseases, systemic neoplasms, organ transplants, HIV infection, or undergoing immunosuppressive therapies. Patients with hypersensitivity to calcipotriol or excipients, as well as those with hypercalcemia, liver failure,

or renal failure, were excluded from the study. Furthermore, patients undergoing conventional photodynamic therapy (C-PDT) treatment or patients who had already initiated daylight photodynamic therapy at the time of study screening were excluded.
Using a simple randomization method (Excel random function), they were randomly divided into two groups of equal number (case and control group).
At the initial visit (time 0), the AKASI score was employed to determine a baseline level of actinic damage in the areas to be treated, and clinical photos were taken. Additionally, the Fitzpatrick skin type was recorded for each patient.
The case group was instructed to undergo a 14-day pretreatment with 0.005% calcipotriol ointment, applied on the areas of scalp and/or face affected by AKs intended for subsequent daylight photodynamic therapy. Calcipotriol was applied once daily, 2 hours before bedtime and removed the following morning washing away with soap and water.
On the fourteenth day, the first session of daylight therapy was initiated. A clinical photo of the areas to be treated was taken. After a mild curettage and cleansing to remove scales and crusts, a thin layer of methyl aminolevulinate (MAL) was applied to the selected areas and the patient was instructed to expose to sunlight within 30 minutes. After 2 hours of sun exposure, MAL was removed with 0.9% saline solution, and SPF50+ sunscreen cream was applied.
Patients were instructed to avoid sun exposure in the following days. Instead, they were advised to apply sunscreen daily to the sun-exposed areas and to wear a protective hat.
Two additional sessions of DL-PDT were then performed at intervals of 10 days to complete the cycle.
For the control group, the procedures were the same as for the cases, but instead of using calcipotriol ointment, they applied a common moisturizing cream to the treatment areas.
At the end of the DL-PDT cycle, patients of both groups were asked to define any discomfort experienced during sun exposure using the Visual Analog Scale (VAS).
Following the photodynamic therapy cycle, patient follow-up appointments were scheduled at 3 and 6 months.
During these follow-up visits, the clinical appearance of the treated areas was assessed using the AKASI scoring system. Iconographic documentation at month 3 and 6 was also collected to monitor the response to therapy (Figure 1 and 2).
In the first analysis, we compared separately the AKASI scores of cases and controls at three times (time 0, 3 months, 6 months) using a paired multiple test.
Subsequently, an oneway ANOVA test for repeated measures was performed to determine whether there were differences in the AKASI score for two different treatments.

Results

All twenty patients completed the study. They were all Caucasian with Fitzpatrick skin types I, II, or III. The average age of the selected patients was 76.1 years, and they were all male.
No difference in skin phototype was detected between the calcipotriol (CAL) group and the controls, as evidenced by a p-value of 0.5 (chi-square test). Similarly, there were no differences in the ages of patients between the cases and controls.
Both groups tolerated the therapy well. The average discomfort score during the sessions assessed with VAS scale was 2 in both the case and control groups.
In the initial analysis the paired multiple test used to compare AKASI scores in cases and controls at different time points (0, 3, 6 months) revealed a significant difference in both cases and controls. The ANOVA oneway test showed statistically significant differences in mean AKASI scores over time for cases ($p < 0.005$), and for controls ($p < 0.005$).
Although no significant difference was found between cases and controls in AKASI at t=0, a significant difference was noted in AKASI at 3 months and AKASI at 6 months post-treatment with CAL plus MAL DL-PDT (Figure 3).

In essence, while a significant decline of AKs was observed in both cases and controls during treatment, comparing cases and controls revealed a greater reduction in AKASI score in CAL group (Figure 4).

Discussion and Conclusions

Calcipotriol is a vitamin D analogue that exhibits several noteworthy activities. It stimulates the production of protoporphyrin IX, an intermediate in the metabolic pathway of porphyrin biosynthesis.[10,11] Since ALA and MAL used in photodynamic therapy also induce an increase in protoporphyrin IX, calcipotriol exerts a synergistic activity in this regard, inducing increased oxidative stress within atypical keratinocytes, ultimately leading to their demise. Additionally, calcipotriol promotes keratinocyte differentiation and induces the expression of thymic stromal lymphopoietin (TSLP) in keratinocytes.[13] TSLP is an epithelium-derived cytokine and the principal regulator of allergic inflammation at the skin level.[13] It is also an inducer of antitumor immunity at the skin barrier by recruiting CD4+ T cells that act as mediators of antitumor immunity.[10,13] Therefore, it can enhance the effectiveness of skin cancer treatment, preventing its development and progression.[10]

Seckin et al. were the first to inquire whether topical 0.005% calcipotriol monotherapy could serve as an alternative treatment for actinic keratoses. The trial compared 0.005% calcipotriol cream to moisturizer cream (placebo) for 12 weeks. The results demonstrated a significant reduction in the total number of AKs in the areas treated with calcipotriol, while no change was observed in the areas treated with placebo.[14]

However, the low concentration of calcipotriol in the therapeutically available formulation (0.005% ointment) leads to a reduction in its effectiveness in inhibiting skin cancer development in mice. Furthermore, 0.005% calcipotriol monotherapy has demonstrated minimal efficacy in actinic keratosis clearance following a 12-week application, coupled with a lack of inflammation at the treatment sites, indicating the absence of immune activation with 0.005% calcipotriol alone.[14]

Building on these results, Cunningham et al. conducted a two-part study to investigate the efficacy of calcipotriol (CAL) in combination with 5-fluorouracil (5-FU) for treating actinic keratoses (AKs).[11] In the first part, they found that CAL-induced TSLP expression in AK keratinocytes led to immune-mediated tumor rejection and reduced tumor growth on murine models. In the second part, they treated 132 patients with either CAL + 5-FU or vaseline + 5-FU. All patients receiving CAL + 5-FU experienced a reduction in AK counts across all treated areas, indicating efficacy in targeting abnormal keratinocytes without affecting normal skin.[11]

A blinded prospective cohort study lead by Rosenberg et al. compared a 4-day course of topical calcipotriol plus 5-FU to a control of vaseline plus 5-FU for treating AK.[10] Squamous cell carcinomas and basal cell carcinomas occurrences were measured at 1, 2, and 3 years post-trial with clinical examination and immunostaining on skin biopsies (taken from the face and scalp). The findings revealed an increase in epidermal resident memory CD4+ lymphocytes (characterized by CD69+ and CD103+ markers) in perilesional skin, indicating the induction of long-lasting T cell immunity within the skin of patients who applied calcipotriol. This immune response corresponded with a reduced risk of squamous cell carcinoma (SCC) development within 3 years of treatment. Notably, the 4-day calcipotriol plus 5-FU treatment showed a stronger association with decreased SCC development on the face and scalp, suggesting a more robust immune induction. This could be attributed to the more severely sun-damaged state of these anatomical sites, resulting in a higher mutational burden that could be more readily targeted by T cells, as well as the higher penetration of topical medications into the skin of the face and scalp compared to that of the upper extremities.[10]

Torezan et al. conducted a randomized controlled clinical trial to compare the long-term 12-month efficacy and safety of conventional methylaminolevulinate (MAL)-PDT with prior application of topical CAL versus standard MAL-PDT for scalp AKs.[12]

CAL-assisted PDT has been demonstrated to be both safe and more effective than conventional MAL-PDT in treating actinic keratoses (AKs) on the scalp. Topical preconditioning of actinic keratosis

with calcipotriol enhances MAL-induced protoporphyrin IX (PpIX) formation compared to the non-pretreated side, resulting in a greater therapeutic effect with photodynamic therapy (PDT).[12]
The management of actinic keratoses is of fundamental importance, as they predispose to cutaneous squamous cell carcinoma. Treatments must aim to reduce the progression of precancerous lesions and prevent their recurrence.[5]
Many treatments are currently available both in hospital settings and for home use. Photodynamic therapy is an effective and well tolerated treatment. Outdoor Day-Light, in particular, is simpler, faster, and less painful compared to conventional photodynamic therapy.
With this study, we aimed to demonstrate how it is possible to enhance this treatment by adding a two-week pre-treatment with 0.005% calcipotriol ointment.
Our results indicate a significant reduction in AKASI scores in patients treated with CAL plus MAL DL-PDT compared to controls during the treatment period. This was evident both from the initial analysis of AKASI scores at different time intervals (0, 3, 6 months) and from the ANOVA analysis of AKASI scores over time.
Additionally, although there was no significant difference in AKASI scores at time t=0, a significant reduction in AKASI scores at 3 and 6 months post-treatment was observed in the CAL plus MAL DL-PDT treated group compared to controls.
The 90% of patients undergoing treatment with calcipotriol tolerated it excellently, reporting only the presence of mild erythema in the treated areas. There was no particular discomfort during the photodynamic therapy sessions compared to the controls.
These data thus describe that, regarding our experience, the application of CAL two weeks before the cycle of daylight photodynamic therapy does not alter the tolerance of the standard treatment.In conclusion, this study demonstrates that with the simple daily domiciliary application of 0.005% calcipotriol ointment followed by a subsequent cycle of three sessions of daylight photodynamic therapy, superior clearance and reduction of actinic keratosis recurrences are achieved compared to standard photodynamic therapy treatment. This outcome suggests the possibility of systematically introducing the use of calcipotriol in conjunction with daylight photodynamic therapy. CAL ointment 0,005% represents a low-cost treatment for the patient and, according to our data, does not significantly alter the tolerance to photodynamic therapy treatment.

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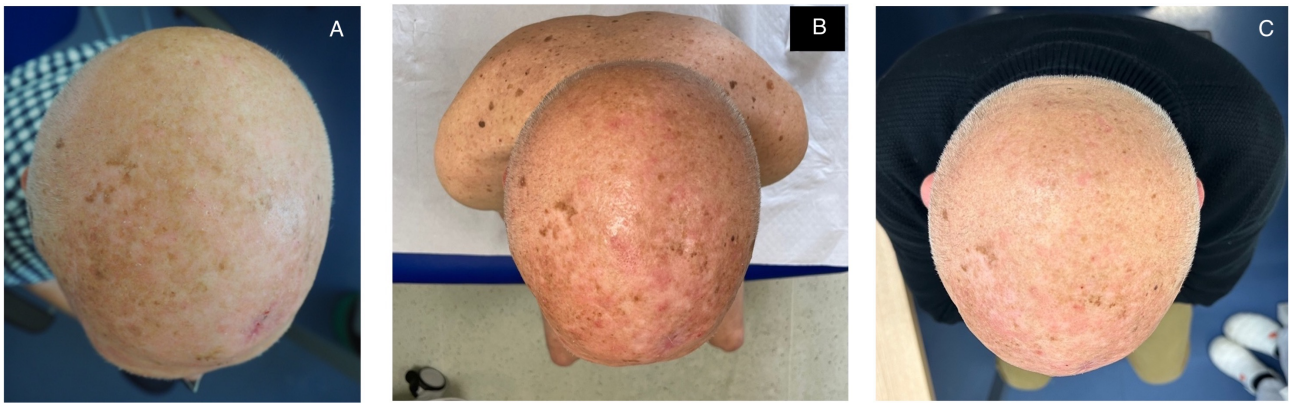

Figure 1. Clinical image of a case group patient at baseline (A), at 3-month follow-up (B), 6-month follow-up (C).

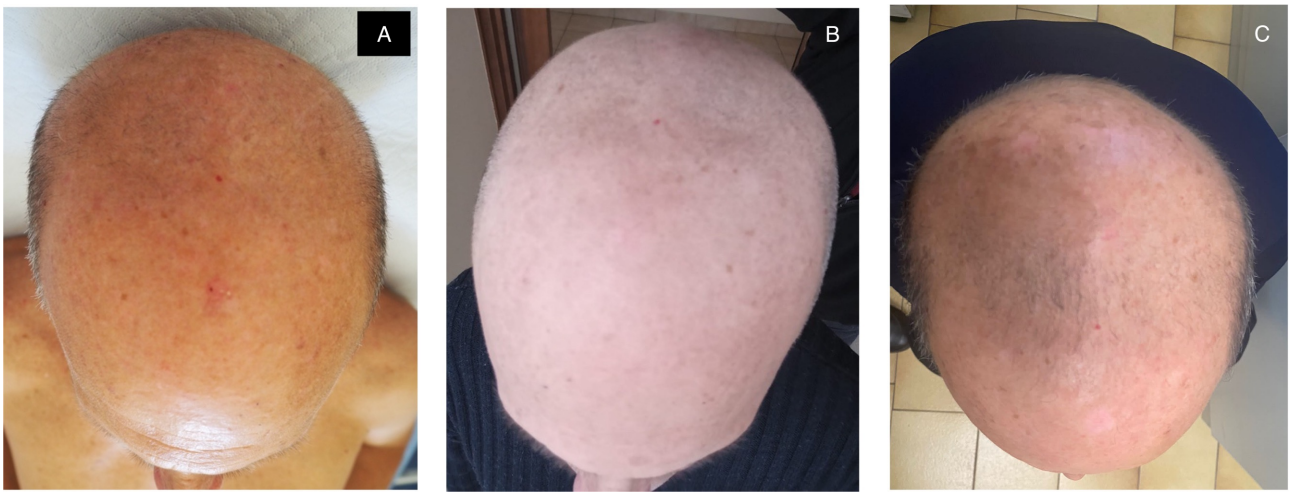

Figure 2. Clinical image of a control group patient at baseline (A), at 3-month follow-up (B), 6-month follow-up (C).

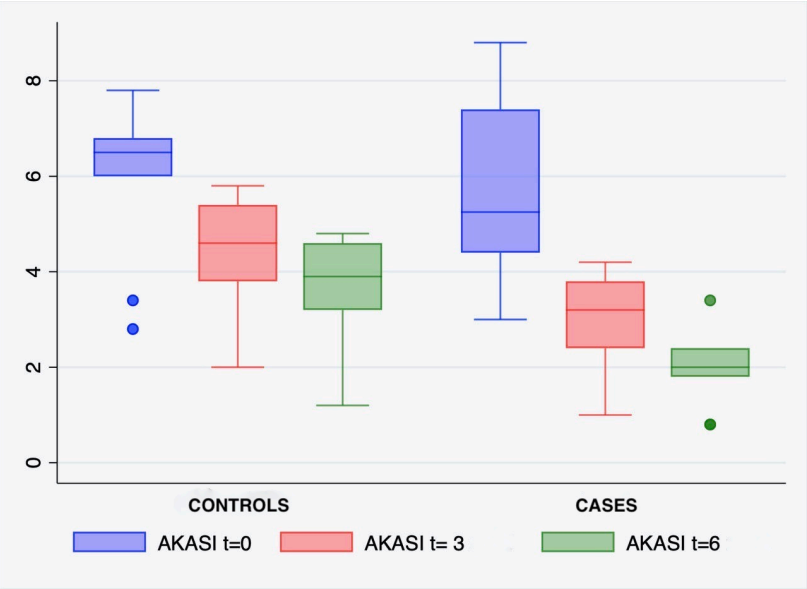

Figure 3. Boxplot representing the AKASI score at baseline (t=0), after 3 months (t=3), and after 6 months (t=6) the end of treatment. While there is no significant difference between cases and controls in AKASI at t=0, there is a significant difference in AKASI at t=3 and AKASI at t=6.

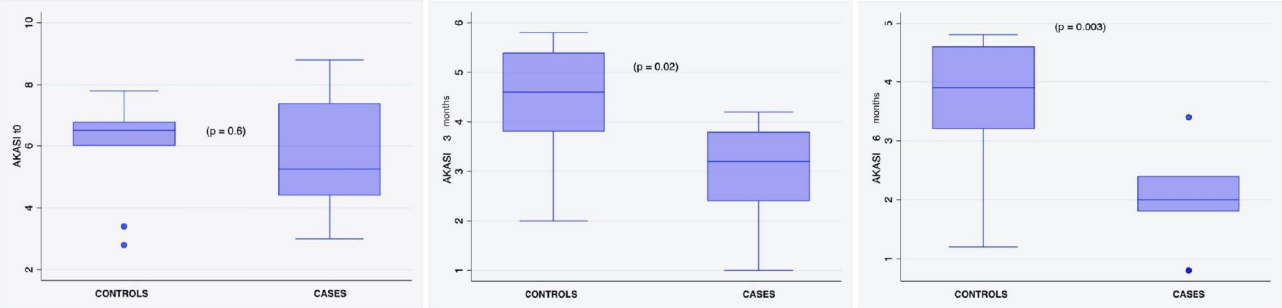

Figure 4. Boxplots representing the AKASI score at baseline t=0, after 3 months, after 6 months the end of treatment.