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Anotācija

Aktīniskās keratozes ir bieži sastopami intraepiteliāli keratinocītu jaunveidojumi, kas ir hroniski saulē bojātas ādas marķieri. Aktīniskās keratozes var tieši progresēt par invazīvu plakanšūnu karcinomu, bet ir jāņem vērā, ka to klātbūtne palielina arī citu ādas audzēju attīstības risku. Tas liecina, ka zināšanām un pētījumiem par aktīnisko keratožu diagnostiku un ārstēšanu ir būtiska klīniska nozīme.

Šī promocijas darba mērķis bija izpētīt dažādus klīniski nozīmīgus aktīnisko keratožu diagnostikas un ārstēšanas aspektus. Mērķis tika sasniegts ar septiņiem uzdevumiem un sešām recenzētām publikācijām.

Pirmajā darba daļā tika skatīts, kuri klīniskie un dermatoskopiskie parametri liecina par slimībai raksturīgajām histoloģiskajām un imūnhistoķīmiskajām pārmaiņām ādā. Lai to panāktu, sākotnēji tika veikts sistemātisks pārskats, lai identificētu tās aktīnisko keratožu klīniskās pazīmes, kas raksturo veidojumus, kuri vēlāk prospektīvos pētījumos progresēja par invazīvām plakanšūnu karcinomām. Šis sistemātiskais literatūras pārskats metodoloģiski tika veikts saskaņā ar *Prisma* vadlīnijām un reģistrēts *Prospero* datubāzē. Darba rezultātā tika secināts, ka ilgstošas un lielas vai saplūstošas aktīniskās keratozes ir visbūtiskākie klīniskie riska faktori tālākai ādas plakanšūnu karcinomas attīstībai no šiem veidojumiem. Papildus tika apkopotas biežākās aktīnisko keratožu dermatoskopiskās pazīmes, kā arī noteikts, kuri no minētajiem imūnhistoķīmiskajiem marķieriem – p53, p63, p16, Ki67, ciklīns D, Bcl-2 un CD31 – ir būtiski aktīnisko keratožu un intraepiteliālo karcinomu paraugos, salīdzinot ar klīniski veselas saulē bojātas ādas paraugiem. Lai to paveiktu, tika apkopoti prospektīvi iegūti aktīnisko keratožu, intraepiteliālu ādas karcinomu un tās pašas vecumgrupas pacientu veselas ādas paraugi no saulei pakļautajām vietām (sejas ādas) un veikti atbilstošie hematoksilīna, eozīna un imūnhistoķīmiskie krāsojumi. Rezultātā tika noskaidrots, ka amorfo masu klātbūtne, p53 krāsojuma intensitāte, Bcl-2 un CD31 marķieru ekspresija atšķiras klīniski veselā hroniski saules bojātā ādā, salīdzinot ar aktīnisko keratožu un intraepidermālu ādas karcinomu paraugiem. Pētījuma tālākā daļā ir sākts analizēt klīnisko un dermatoskopisko parametru saistību ar histoloģiskām un imūnhistoķīmiskām pārmaiņām. Rezultātā ir iegūts, ka dermatoskopiski tipiskas aktīniskās keratozes, tostarp ar raksturīgo mazo starpfolikulāro asins kapilāru klātbūtni, ir biežāk redzamas, ja histopatoloģiskajā izmeklēšanā ir amorfas masas. Turklāt dermatoskopiski tipiskām aktīniskajām keratozēm ir zemāka CD31 marķiera ekspresija, savukārt folikulu trūkums bija saistīts ar palielinātu Bcl-2 marķiera subepidermālo ekspresiju. Šie rezultāti skaidrojami ar hroniskā ultravioletā starojuma nozīmi aktīnisko keratožu attīstībā, un tie izceļ neoangioģenēzes un subepidermālās iekaisuma šūnu infiltrācijas

nozīmi aktīnisko keratožu progresēšanā līdz plakanšūnu karcinomai. Turklāt šie rezultāti uzsver dermatoskopijas nozīmi tādu veidojumu atlasē, kuriem ir lielāka smaguma pakāpe un tāpēc var būt nepieciešama rūpīgāka uzraudzība vai agresīvāka terapija.

Darba otrajā daļā tika pētīti ar aktīnisko keratožu ārstēšanu saistīti aspekti. Pirmkārt, šī darba ietvaros tika dermatoskopiski sekots aktīnisko keratožu dermatoskopiskajām pārmaiņām ārstēšanas laikā, tostarp ārstēšanas radītā iekaisuma fāzē. Tas ir būtiski, jo bieži diagnoze tiek pamatota ar dermatoskopiskām pazīmēm, savukārt, ja dermatoskopiskās pazīmes ir mainīgas, tas var ietekmēt diagnostisko procesu. Pētījuma rezultātā tika secināts, ka baltās spīdīgās strēles ir mainīgas dermatoskopiskās struktūras, kuras var izzust terapijas radītā iekaisuma laikā, var izzust pēc lokālās terapijas un dažkārt var parādīties pirmo reizi pēc ārstēšanas. Turklāt, veicot dinamisko novērošanu, mēs varējām vizualizēt un ziņot par retu klīnisko gadījumu, kurā aktīniskās keratozes ir vienā lokalizācijā ar virspusēju bazālo šūnu karcinomu un šī karcinoma ir dermatoskopiski diagnosticējama tikai pēc aktīnisko keratožu terapijas.

Turpinot terapijas aspektu izpēti, tika salīdzināta smagu aplikācijas vietu reakciju sastopamība starp dažādām aktīnisko keratožu lauka ārstēšanas metodēm. Ir zināms, ka aktīnisko keratožu ārstēšana sniedz daudz priekšrocību pacientiem, tomēr lauka ārstēšanas metodes rada iekaisuma reakcijas, kuras pacientiem bieži ir grūti pieņemamas. Tāpēc tika veikts literatūras apskats un secināts, ka smagas intensitātes medikamenta radītas aplikācijas vietas reakcijas ir bieži sastopamas, īpaši terapijā ar imihimodu. Vienīgā terapeitiskā metode ar zemu smagu lokālo reakciju izplatību bija dienasgaismas fotodinamiskā terapija. Ārstēšanas pārtraukšana lokālo reakciju dēļ ir bieža, lai gan visaugstākais ārstēšanas pārtraukšanas biežums lokālo reakciju dēļ tika novērots pētījumos ar visilgākajām ārstēšanas shēmām, piemēram, ar diklofenaku, nevis pētījumos, kuros ziņots par visaugstāko smagu lokālo reakciju sastopamību. Šie secinājumi ir klīniski būtiski, nosakot lokālo terapiju aktīnisko keratožu pacientiem.

Atslēgvārdi: aktīniskā keratoze, intraepiteliālā karcinoma, plakanšūnu karcinoma, ādas vēzis, dermatoskopija, UV starojums, aplikācijas vietas reakcijas.

Abstract

Diagnostic and Treatment Aspects of Actinic Keratoses

Actinic keratoses (AK) are common keratinocyte intraepithelial neoplastic disorders that serve as a general marker of chronic sun damage. These lesions can directly progress into invasive squamous cell carcinoma (SCC), while simultaneously, their presence serves as a general marker of all skin cancer development risks. Therefore, knowledge in diagnosing and treating AK is of great clinical relevance.

The aim of this Thesis was to explore different clinically relevant aspects of diagnosing and treating AK. The aim was reached with seven tasks and six peer-reviewed publications.

The initial part of the study focused on identifying clinical and dermatoscopic parameters that are indicative of histological and immunohistochemical alterations in the skin, typical of the disease. This was accomplished through a systematic review aimed at pinpointing the clinical characteristics of AK that were known to progress into invasive SCCs, as evidenced in prospective studies. The review followed the Prisma guidelines and was registered in the Prospero database. As a result, long-standing and large or merging AK were concluded to be the most important clinical risk factors for the development of SCC. Furthermore, the study summarized the most prevalent dermatoscopic features of AK and evaluated the significance of various immunohistochemical markers – namely p53, p63, p16, Ki67, cyclin D1, Bcl-2, and CD31. This evaluation aimed to discern their expression in samples of AK and intraepithelial carcinomas as opposed to samples of clinically normal but sun-damaged skin. To accomplish this objective, the study collected AK and intraepithelial carcinoma samples, as well as healthy skin samples from sun-exposed facial areas, from patients within the same age cohort. These samples were then stained with haematoxylin and eosin, along with specific immunohistochemical markers. The findings revealed distinct differences in the presence of amorphous masses and the intensity of staining for p53. Moreover, there was a variation in the expression of Bcl-2 and CD31 markers between clinically healthy, chronically sun-damaged skin and the samples of AK and intraepidermal carcinomas. In a subsequent phase of the research, an analysis was conducted to elucidate the correlation between identified clinical and dermatoscopic parameters and the observed histological and immunohistochemical alterations. This analysis revealed that dermatoscopically typical AK, particularly those featuring the hallmark small interfollicular blood capillaries, were more likely to be associated with the presence of amorphous masses in histopathological samples. Additionally, these typical AKs exhibited a lower expression of the CD31 marker. Conversely, the absence of follicular structures correlated with an increased expression of the Bcl-2 marker in the subepidermal

layers. The identified associations underscore the significance of neoangiogenesis and subepidermal inflammatory cell infiltration in the recognized progression from AK to SCC. Furthermore, these findings emphasize the utility of dermatoscopy as a tool for selecting lesions that exhibit greater severity and may therefore require closer monitoring or more aggressive management. The investigation into the correlation between dermatoscopic and histopathological findings is set to continue in future studies.

In the second part of the Thesis, aspects concerning the treatment of AK were investigated. The research initially tracked the dermatoscopic alterations in AK throughout the therapeutic process, particularly noting the changes during the treatment-induced inflammatory phase. This aspect was crucial, as dermatoscopic signs often form the basis of diagnosis, and variability in these signs could influence diagnostic accuracy. The study found that shiny white streaks, which are considered variable dermatoscopic structures, may vanish during inflammation caused by treatment, may resolve post-topical therapy, or may even emerge de novo post-treatment. Additionally, through dynamic monitoring, a rare clinical instance was documented where AK coexisted with a superficial basal cell carcinoma (BCC). Intriguingly, the BCC was only dermatoscopically identifiable following the treatment of the AK, highlighting the complex interplay between dermatoscopic visibility and therapeutic intervention.

Continuing with the exploration of therapeutic aspects the occurrence of severe application site reactions across various field treatments for AK were compared. While such treatments are beneficial, they can elicit inflammatory responses that many patients find challenging to endure. A comprehensive review of the existing literature was conducted, leading to the conclusion that severe drug-induced application site reactions are notably prevalent, particularly with imiquimod therapy. Daylight photodynamic therapy emerged as the only method exhibiting a low frequency of severe local reactions. Discontinuation of treatment due to adverse local reactions was a frequent occurrence, with the longest treatment protocols, such as those involving diclofenac, showing the highest discontinuation rates – not necessarily those with the greatest incidence of severe reactions. These insights hold considerable clinical importance for the prescription of topical therapies to patients with AK.

Keywords: actinic keratosis, squamous cell carcinoma, skin cancer, progression, dermatoscopy, dermatoscopic-immunohistochemical correlations, UV damage, application site reactions.

Satura rādītājs

Darbā izmantotie saīsinājumi

AK	aktīniskā keratoze
Bcl-2	B šūnu limfomas 2 (*B-cell lymphoma* 2) gēna proteīns
CD31	klasteru diferenciācijas marķieris (*cluster of differentiation*) 31
Ki67	kodola proteīns Ki67
p16	proteīns p16
p53	proteīns p53
p63	proteīns p63
UV	ultravioletā gaisma

Ievads

Aktīniskās keratozes (AK) ir vienas no visbiežāk sastopamiem ādas bojājumu veidiem kā vispārējā, tā dermatoloģiskajā praksē. Klīniski tās parasti ir redzamas kā multiplas sārtas makulas, papulas vai plātnītes ar virsmas zvīņu uz hroniski saulē bojātas ādas. Lielākoties aktīniskās keratozes skar gados vecākus cilvēkus ar gaišu ādu (Dziunycz et al., 2018; Reinehr and Bakos, 2019; Schmitz et al., 2018; Steuer et al., 2020; Tizek et al., 2019). Vislielākā AK klīniskā nozīme ir to iespējamā progresēšana par invazīvu plakanšūnu karcinomu, kas ir otrs izplatītākais ādas vēža veids (Stratigos et al., 2020b), lai gan lielai daļai pacientu tieši kosmētiskais izskats ir iemesls saņemt ārsta konsultāciju. Morfoloģiski AK ir intraepidermālas neoplāzijas, kas tiek uzskatītas par plakanšūnu karcinomas *in situ* stadiju un ir sākumposms invazīvu plakanšūnu karcinomu attīstībai (Reinehr and Bakos, 2019; Rowert-Huber et al., 2007). Plakanšūnu karcinomas var attīstīties tieši no AK vai kā jauns bojājums uz blakus esošās hroniski saulē bojātās ādas, jo AK klātbūtne palielina visu turpmāko ļaundabīgo ādas audzēju risku, īpaši plakanšūnu karcinomu (Guorgis et al., 2020). Lai gan ir sarežģīti aprēķināt precīzu katras AK progresēšanas risku, nesenais *Madani* et al. (Madani et al., 2021) pētījums parādīja, ka kumulatīvā ādas plakanšūnu karcinomu sastopamība AK pacientiem sasniedza 17,1 % 10 gadu laikā pēc diagnozes noteikšanas. Ir klīniski būtiski, ka AK un plakanšūnu karcinomu diferenciāldiagnostika sākotnējās stadijās ir sarežģīta, un pētījumos ir dati, ka 1,5 % no sertificēta dermatologa klīniski diagnosticētām AK bija plakanšūnu karcinomas ar virspusēju invāziju histoloģiskajā novērtējumā (Ehrig et al., 2006). Visu ādas bojājumu, tostarp AK un plakanšūnu karcinomu, diagnostiku atvieglo dermatoskopija. Dermatoskops ir bieži sastopams un viegli lietojams instruments dermatoloģijas praksē, un nesen veikts sistemātisks pārskats parādīja šīs metodes augsto precizitāti – jutīguma līmeni līdz 98,7 % un specifiskumu līdz 95 %, ja AK tiek diagnosticēta, pateicoties dermatoskopiskajām pazīmēm (Huerta-Brogeras et al., 2012; Valdés-Morales et al., 2020). Turklāt iepriekš ir publicēts, kā mainās AK dermatoskopiskās pazīmes, progresējot par invazīvu plakanšūnu karcinomu (Zalaudek et al., 2012). Lai gan vairākos pētījumos ir novērtētas AK un plakanšūnu karcinomu klīnisko, dermatoskopisko un histoloģisko pazīmju korelācijas (Lee et al., 2019; Papageorgiou et al., 2022; Rstom et al., 2022), rezultāti nav vienoti un trūkst pētījumu, kas novērtētu arī imūnhistoķīmiskās īpašības.

Atbilstoši vadlīnijām AK ārstēšana ar retiem izņēmumiem ir indicēta visiem pacientiem, lai novērstu progresiju. Terapija var būt vērsta uz atsevišķiem veidojumiem vai visu AK lauku, kas nozīmē arī bojāto, klīniski neredzamo šūnu ārstēšanu starp redzamajiem AK veidojumiem. Visas ārstēšanas iespējas, izņemot ķirurģisku veidojumu ekscīziju, ir vērstas uz atipisko

keratinocītu iznīcināšanu, tā izraisot īslaicīgas iekaisuma reakcijas ar dažādu smaguma pakāpi. Ar ārstēšanu saistīto aplikācijas vietu reakciju apspriešana pirms terapijas var izraisīt ārstēšanas sākšanas aizkavēšanos. Ārstēšanas laikā šādas reakcijas izraisa diskomfortu (Eisen et al., 2021; Neri et al., 2019) un ietekmē ar veselību saistīto dzīves kvalitāti (Hanke et al., 2016). *Strohal* et al. (Strohal et al., 2012) pētījums parādīja, ka 19,4 % pacientu, kuri tika ārstēti ar lokālu imihimoda 5 % krēmu, veica neplānotas vizītes, jo viņiem bija bažas par aplikācijas vietu reakcijām. Turklāt aplikācijas vietu reakcijas izraisa nelīdzestību, tādējādi, iespējams, samazinot ārstēšanas efektivitāti (Stockfleth et al., 2015). Lokālā terapija ir indicēta lielākajai daļai pacientu ar AK un lauka kancerizāciju, jo tā ir viegli lietojama, ļoti efektīva, ietaupa ārsta laiku un ir vienīgā ārstēšanas metode, kas vērsta uz visu zonu. Šajā darbā tika pētīti ar AK ārstēšanu saistīti faktori, kā smagu aplikācijas vietu reakciju izplatība, dermatoskopisko pazīmju izmaiņas terapijas laikā un pēc tās.

Darba mērķi

1. Noteikt klīniskās, dermatoskopiskās, histopatoloģiskās un imūnhistoķīmiskās pazīmes, kas raksturo aktīniskās keratozes un intraepidermālas plakanšūnu karcinomas.
2. Izvērtēt, kā mainās aktīnisko keratožu dermatoskopiskās pazīmes terapijas radītā iekaisuma laikā un pēc terapijas.
3. Noteikt aktīnisko keratožu terapijas radīto smago lokālo ādas reakciju sastopamību un ar to saistīto terapijas pārtraukšanas biežumu.

Darba uzdevumi

Promocijas darba mērķu sasniegšanai tika izvirzīti šādi uzdevumi:

1. Sistemātiska pārskata veidā apkopot literatūras datus par prospektīvos pētījumos novērotām klīniskajām pazīmēm, kas raksturo aktīniskās keratozes, kuras progresēja par plakanšūnu karcinomām.
2. Apkopot biežākās publicētās dermatoskopiskās pazīmes, kas raksturīgas sejas ādas aktīniskajām keratozēm, intraepidermālajām ādas karcinomām un plakanšūnu karcinomām, un sniegt šo pazīmju skaidrojumus.
3. Izpētīt un novērtēt, kuriem no minētajiem imūnhistoķīmiskajiem marķieriem – p53, p63, p16, Ki67, ciklīns D, Bcl-2 un CD31 – ir atšķirīga ekspresija klīniski veselā, saulē bojātā ādā, salīdzinot ar aktīniskās keratozes un intraepidermālās karcinomas ādas paraugiem.
4. Izpētīt un novērtēt, kuras no klīniskajām un dermatoskopiskajām aktīnisko keratožu un intraepidermālo karcinomu pazīmēm ir biežāk sastopamas, kad konstatē aktīnisko

keratožu progresijai raksturīgās patohistoloģiskās pārmaiņas un izmantoto imūnhistoķīmisko marķieru – p53, p63, p16, Ki67, ciklīns D, Bcl-2 un CD31 – ekspresijas izmaiņas.

5. Izpētīt un novērtēt, kā lokālās terapijas radītais iekaisums maina dermatoskopiskās aktīnisko keratožu pazīmes.
6. Izvērtēt aktīnisko keratožu dermatoskopiskās pazīmes pirms un pēc terapijas.
7. Apkopot literatūras datus par aktīnisko keratožu terapijas radīto smago lokālo ādas reakciju sastopamību un ar to saistīto terapijas pārtraukšanas biežumu.

Darba hipotēzes

1. Pēc aktīnisko keratožu klīniskajām un dermatoskopiskajām pazīmēm ir iespējams paredzēt histopatoloģiskās pazīmes un imūnhistoķīmisko marķieru – p53, p63, p16, Ki67, ciklīns D, Bcl-2 un CD31 – ekspresiju.
2. Aktīnisko keratožu dermatoskopiskās pazīmes kļūst mazāk izteiktas terapijas radītā iekaisuma fāzē, un tās pilnībā izzūd pēc sekmīgas terapijas.
3. Aktīnisko keratožu ārstēšana bieži izraisa smagas lokālas reakcijas medikamenta aplikācijas vietās, kā rezultātā pacienti pārtrauc ārsta nozīmēto terapiju.

Darba novitāte

Šis darbs sastāv no vairākiem pētījumiem, un katrs atbild uz savu pētniecības jautājumu. Pirmkārt, atjauninot iepriekš 2006. gadā publicēto *Quaedvlieg* et al. (Quaedvlieg et al., 2006) pētījumu, sistemātiskā pārskata veidā tika identificētas tās klīniskās pazīmes, kas raksturo AK ar augstāku risku progresēt par invazīvu plakanšūnu karcinomu. Proti, tika iegūts, ka publicētos prospektīvos pētījumos no ilgstošām un lielām (virs 1 cm diametrā) vai saplūstošām AK biežāk vēlāk attīstījās invazīva karcinoma. Otrkārt, šī darba ietvaros tika novērtēta septiņu imūnhistoķīmisko marķieru (p53, p63, p16, Ki67, ciklīns D, Bcl-2 un CD31) ekspresija gados vecāku cilvēku klīniski veselos hroniski saulē bojātas ādas paraugos (kontroles grupa) un AK/intraepidermālo karcinomu grupā, tādējādi identificējot slimībai specifiskās izmaiņas. Īpaši izceļams ir lielais vienlaikus noteikto marķieru skaits. Darba rezultātā tika iegūts, ka amorfo masu izplatība, p53 krāsojuma intensitāte, Bcl-2 un CD31 ekspresija klīniski veselā saulē bojātā ādā atšķiras, salīdzinot ar ādas paraugiem, kuros attīstījušās AK un intraepidermālās karcinomas. Pētījumu turpinot, iegūtie rezultāti tika izmantoti, lai savstarpēji salīdzinātu katra parauga klīniskos, dermatoskopiskos, histopatoloģiskos un imūnhistoķīmiskos parametrus. Šis pētījums ir unikāls arī no tā aspekta, ka pacienti tika iekļauti prospektīvi un ka tika salīdzināta dermatoskopisko pazīmju klātbūtne ar imūnhistoķīmisko marķieru ekspresiju, kas literatūrā joprojām ir salīdzinoši reti, lielākoties gadījumu aprakstos. Rezultātā tika identificēts, ka

dermatoskopiski tipiskas AK, tostarp veidojumi ar raksturīgajiem maziem zarotiem asins kapilāriem, histopatoloģijā biežāk uzrāda amorfas masas. Dermatoskopiski tipiskām AK bija zemāka CD31 ekspresija un folikulu trūkums bija saistīts ar augstāku Bcl-2 subepidermālo ekspresiju. Šādi atklājumi sakrīt un papildina pašreizējās zināšanas par marķieriem, kas iesaistīti keratinocītu karcinomu progresijā.

Otrā darba daļā tika pētīti ar AK ārstēšanu saistītie aspekti. Pirmkārt, dermatoskopisko pazīmju pārmaiņas terapijas laikā un pirmo reizi tika ziņots par balto, spīdīgo strēļu variabilitāti. Papildus – literatūrā ir reti ziņojumi par vienā lokalizācijā esošu AK un bazālo šūnu karcinomu, bet, fotodokumentējot AK terapijas laikā, tika iegūts, ka, izārstējot epidermā lokalizētos AK veidojumus, ir iespēja diagnosticēt dziļāk esošos veidojumus – šajā gadījumā bazālo šūnu karcinomu – un ziņot par to. Īstenojot šo darbu un, cik autorei zināms, pirmo reizi 2018. gadā Latvijā vairākiem pacientiem tika veikta dienas gaismas fotodinamiskā terapija un apkopoti tās rezultāti. Savukārt noslēdzošajā daļā, papildus pētot ar aplikācijas vietu reakcijām saistītos aspektus, tika iegūts, ka pacienti biežāk pārtrauc terapijas, kam ir ilgstoša lietošanas shēma, nevis tās, kuras rada izteiktākās reakcijas. Minētais fakts pastiprina konsultācijas nozīmību reakciju skaidrošanā, tā uzlabojot līdzestību un iegūstamo terapijas efektivitāti.

Diskusija

Ultravioletās (UV) gaismas iedarbība ir būtiskākais ādas vēzi izraisošais apkārtējās vides faktors. UV gaismas rezultātā saulei pakļautās vietas, piemēram, sejas āda, ir visbiežākā ādas vēža lokalizācija (Reinehr and Bakos, 2019; Subramaniam et al., 2017; van der Pols et al., 2006a). AK ir bieži sastopami UV gaismas izraisīti ādas veidojumi, kas lielākoties atrodami uz gaišādainu cilvēku hroniski saulē bojātās ādas. Klīniski būtiska ir šo veidojumu spēja attīstīties par invazīvu plakanšūnu karcinomu, kas ir otrs biežākais ādas audzēju veids. Kaut arī daļa autoru uzskata, ka nav iespējams noteikt, no kuras AK attīstīsies invazīva plakanšūnu karcinoma (Stockfleth et al., 2012), un ka šie veidojumi vairāk ir vispārējs ādas audzēju riska marķieris, nevis tieši priekšteči (Stratigos et al., 2020a), *Quaedvlieg* et al. pētījums, kas tika publicēts 2006. gadā, identificēja vairākus klīniskos kritērijus, kuri saistīti ar AK progresēšanu par plakanšūnu karcinomu, un tika ieviests saīsinājums "IDRBEU". Šajā akronīmā "I" apzīmē iekaisumu/indurāciju; "D" – diametru > 1 cm; "R" – strauju palielināšanos izmērā; "B" – asiņošanu; "E" – eritēmu un "U" – izčūlojumu. Papildu mazie klīniskie kritēriji, kas tika identificēti *Quaedvlieg* et al. pētījumā, bija sāpes, palpējamība, hiperkeratoze, nieze un pigmentācija (Quaedvlieg et al., 2006). Šī promocijas darba pirmajā daļā tika veikts sistemātisks literatūras pārskats, lai atjauninātu *Quaedvlieg* et al. pētījuma rezultātus, balstoties uz prospektīviem pētījumiem, kas publicēti pēc 2005. gada. Pārskats tika veidots atbilstoši sistemātisko pārskatu veidošanas vadlīnijām un pirms pētījuma sākšanas reģistrēts *Prospero* datubāzē, kā arī veikts atbilstoši *Prisma* vadlīnijām, kā to nosaka vispārpieņemtā prakse. Rezultātā tika iegūts, ka ilgstoši esošas un lielas (> 1 cm) vai saplūstošas AK ir tās, no kurām visbiežāk pēc tam attīstījās invazīvas plakanšūnu karcinomas augsta riska populācijās. Šāds rezultāts, identificējot divus klīniski viegli nosakāmus parametrus, ļauj novērtēt pacienta risku papildus kopējai smaguma pakāpei un izvēlēties veidojumus, kuru terapija būtu nepieciešama primāri, ja visus veidojumus nav iespējams ārstēt jau sākotnēji.

Papildus būtiskāko klīnisko pazīmju identificēšanai tika apkopotas biežākās sejas nepigmentēto AK un intraepiteliālo karcinomu dermatoskopiskās pazīmes, kas kopā ar šo pazīmju skaidrojumiem un atbilstošo pazīmju attēliem 2022. gadā tika publicētas kā nodaļa grāmatā ar nosaukumu *Dermatoscopy* (skat. publikāciju sarakstu).

Tālāk promocijas darba ietvaros tika salīdzinātas histopatoloģiskās pazīmes un izmantoto imūnhistoķīmisko marķieru – p53, p63, p16, Ki67, ciklīns D, Bcl-2 un CD31 – ekspresija prospektīvi iegūtiem normālas hroniski saulē bojātas ādas paraugiem un AK/intraepiteliālo karcinomu paraugiem. Iekļautajiem kontroles grupas pacientiem vecums statistiski neatšķīrās no pētāmās grupas, lai mazinātu iespējamo vecuma un kumulatīvā UV

starojuma ietekmi uz imūnhistoķīmisko marķieru ekspresiju, jo iepriekš ir pierādīts, ka šie faktori ietekmē imūnhistoķīmiskos marķierus (Bakshi et al., 2020; Khodaeiani et al., 2013; Nasiri et al., 2021). Šajā pētījumā tika atklātas atšķirības amorfo masu izplatībā, p53 krāsošanās intensitātē, Bcl-2 un CD31 ekspresijā starp pētījuma un kontroles grupām. Konkrētāk, pirmkārt, tika iegūts, ka amorfās masas bija vērojamas lielākajā daļā AK/intraepidermālo karcinomu gadījumu un nevienā no kontroles biopsijām. Ir zināms, ka ādas kolagēna aizstāšana ar amorfām elastīga materiāla masām raksturo smagu saules elastozi un ir pārmērīga kumulatīvā UV bojājuma sekas (Karagas et al., 2007). Šis rezultāts atbilst UV starojumam kā galvenajam AK cēlonim un iepriekšējiem pētījumiem, kuros ir konstatēta smagas pakāpes saules elastoze vairumā ādas plakanšūnu karcinomu gadījumu (Corbalán-Vélez et al., 2010; Karagas et al., 2007).

Otrkārt, tika konstatēta augstāka p53 krāsošanās intensitāte AK/intraepidermālo karcinomu grupā, kas atbilst iepriekš publicētiem pētījumiem un p53 proteīna lomai agrīnajos plakanšūnu karcinomas attīstības posmos, ļaujot šūnām apiet apoptozes procesu (Bakshi et al., 2020; Berhane et al., 2002; Javor et al., 2021; Piipponen et al., 2021). Vienlaikus netika vērotas atšķirības starp p53 ekspresijas apjomu un sadalījumu epidermas slāņos starp abām grupām. Līdzīgi pētījumiem, ko veica *Neto* et al. (Neto Pimentel et al., 2013) un *Piipponen* et al. (Piipponen et al., 2020), ne visi iekļautie AK paraugi uzrādīja pozitīvu p53 krāsojumu. Vairāk nekā 5 % imūnreaktivitāte pret p53 tika novērota 75,8 % AK/intraepidermālo karcinomu paraugu, un krāsojums tika uzskatīts par spēcīgu 44,8 % gadījumu. No literatūras datiem ir zināms, ka ievērojami augstāka p53 krāsošanās ir novērota, pieaugot pacienta vecumam, un saulei pakļautās vietās, savukārt p53 ekspresija ādā samazinās, katru dienu lietojot saules aizsarglīdzekļus (van der Pols et al., 2006b). Šo faktoru ietekme tika mazināta, gan pētījuma, gan kontroles grupas paraugus ņemot no sejas ādas un iekļaujot pacientus, kuru vecums statistiski neatšķīrās.

Treškārt, pētījuma rezultātā tika iegūts, ka AK un intraepidermālo karcinomu paraugiem bija augstāka Bcl-2 ekspresija epidermas slāņos un dermā, salīdzinot ar kontroles grupu. Bcl-2 ir antiapoptotisks proteīns, kas atrodas mitohondriju membrānā. Tā funkcijas maiņa un pārmērīga ekspresija inhibē apoptozi, kas var veicināt vēža attīstību. Savukārt normālā ādā Bcl- 2 pozitīvas bazālās šūnas ir rezervuārs plakanšūnu epitēlija atjaunošanai, un saulei pakļautā ādā Bcl-2 novērš UV izraisītu šūnu nāvi (Hussein and Ahmed, 2022; Onder et al., 2019). Paaugstināta Bcl-2 ekspresija ir iepriekš novērota AK, un Bcl-2 eksprimējošu audzēja šūnu ir ievērojami vairāk plakanšūnu karcinomās, salīdzinot ar AK (Berhane et al., 2002; Woo et al., 2017). Turklāt Bcl-2 var krāsot arī dermas iekaisuma šūnu infiltrātu, un iekaisums ir saistīts ar AK progresēšanu par ļaundabīgu plakanšūnu karcinomu, veidojot skābekļa brīvos

radikāļus, veicinot imūnās atbildes reakciju, šūnu transformāciju, proliferāciju, invāziju, angioģenēzi un metastāzes (Farshchian et al., 2017; Singh et al., 2019; Woo et al., 2017). Iepriekš ir publicēti dati par pakāpenisku Bcl-2 ekspresijas pieaugumu no asimptomātiskas AK caur iekaisušu AK līdz plakanšūnu karcinomai, kas pamatotu AK progresēšanu par invazīvu plakanšūnu karcinomu caur iekaisuma fāzi (Berhane et al., 2002). Mūsu rezultāti parādīja, ka gan Bcl-2 subepidermālais infiltrāts, gan CD31 (jutīgākais un specifiskākais endotēlija marķieris parafīna sekcijās (Stuart, 2013)) ir palielināts AK/intraepidermālu karcinomu paraugos, salīdzinot ar kontrolēm, kas kopumā atbilst iepriekš minētajai iekaisuma lomai AK patoģenēzē.

Kā viena no šīs promocijas darba sadaļas kvalitātēm ir jāatzīmē visu pētījumā iekļauto ādas biopsiju ieguve no sejas ādas.

Jāpiemin, ka pētījumā lielākoties tika iekļautas sievietes. Kaut arī lielākajā daļā AK apraksta ir minēts, ka AK biežāk tiek diagnosticētas vīriešiem un, ņemot vērā plikpaurības izplatību vīriešu vidū, no tās izriet lielāks saulei pakļautās ādas apjoms, arī citos pētījumos ir bijis vairāk sieviešu (Kohl et al., 2017; Lee et al., 2019; Madani et al., 2021). Turklāt mūsu izlasē iekļauto pacientu vidējais vecums bija 78,1 gads, tāpēc sieviešu pārsvars skaidrojams ar vidējo paredzamo mūža ilgumu Latvijā, kas 2021. gadā sasniedza 68,2 gadus vīriešiem un 77,9 gadus sievietēm (Centrālā statistikas pārvalde, 2021).

Turpinot analizēt iegūtās histopatoloģiskās un imūnhistoķīmisko marķieru pārmaiņas saistībā ar katram veidojumam atbilstošajām dermatoskopiskajām pazīmēm, tika iegūts, ka dermatoskopiski klasiskām AK biežāk ir redzamas amorfās masas un ir mazāka CD31 marķiera ekspresija, kas norāda uz mazāku asins kapilāru klātbūtni. Papildus tam Bcl-2 subepidermālā ekspresija bija saistīta ar folikulu zudumu. Šīs sākotnējās korelācijas izceļ asins kapilāru pārmaiņas un folikulu zudumu kā būtisku soli AK progresēšanai par plakanšūnu karcinomu, turklāt šīs pazīmes ir iespējams vizualizēt neinvazīvi ar dermatoskopa palīdzību.

Dermatoskops ir ērti lietojama, rokā turama ierīce, kas nodrošina desmitkārtīgu palielinājumu un novērš atstarojumu no ādas virsmas, tā vizualizējot morfoloģiskās struktūras, kas nav redzamas ar neapbruņotu aci (Pan et al., 2008). Ir pierādīts, ka dermatoskopijas izmantošana uzlabo gan pigmentētu, gan nepigmentētu ādas veidojumu diagnostisko precizitāti un dažkārt sniedz būtisku informāciju par veidojuma prognozi, izmantojot zināšanas par dermatoskopiski histopatoloģiskajām korelācijām (Sinz et al., 2017). Biežākās dermatoskopiskās AK pazīmes ir baltas vai dzeltenas krāsas virsmas zvīņas, sarkans pseidotīklojums, ko veido perifolikulāra eritēma, bieži vien kopā ar lineāri viļņveidīgām un/vai zarotām starpfolikulārām teleangiektāzijām, izceltas folikulu atveres, kuras redzamas kā balts

aplis ap folikulāro atveri ar dzeltenīgu keratīna korķi folikula centrā, un rozetes pazīme (Lee et al., 2014; Zalaudek et al., 2006).

Šajā promocijas darbā tika skatīta gan atsevišķo veidojumu dermatoskopisko pazīmju saistība ar histoloģiskajām un imūnhistoķīmiskajām pārmaiņām, kā iztirzāts iepriekš, gan atsevišķu veidojumu dermatoskopisko pazīmju mainība terapijas laikā. Šajā otrajā promocijas darba daļā pacientiem ar digitālo dermatoskopiju tika uzņemti klīniskie un dermatoskopiskie attēli pirms terapijas, terapijas radītā iekaisuma fāzē un pēc terapijas. Pacienti tika sadalīti divās grupās atkarībā no rekomendētā terapijas veida – terapijas ar lokālu 5 % 5-fluoruracila krēmu vai dienas gaismas fotodinamisko terapiju ar metilaminolevulinātu (Metvix®, Galderma). Pētījuma rezultātā tika novērots, ka baltās, spīdīgās strēles ir biežāk sastopamas AK nekā iepriekš publicētajos rakstos (Balagula et al., 2012; Liebman et al., 2012) un ka tās ir mainīgas dermatoskopiskas struktūras, kas var izzust terapijas radītā iekaisuma laikā un pirmo reizi parādīties pēc terapijas. Baltās, spīdīgās strēles ir zināmas arī kā kristāliskās struktūras un ir baltas, perpendikulāras, dažus milimetrus garas līnijas, kas redzamas tikai polarizētās gaismas dermatoskopijā (Kittler et al., 2016). Šīs struktūras, ko uzskata par dermatoskopisku dermas fibrozes pazīmi, izraisa sabiezējušu hialīna šķiedru kūlīšu polarizācija (Haspeslagh et al., 2016; Pizzichetta et al., 2014), un ir ziņots par to sastopamību dažādos ādas veidojumos, tostarp AK (Balagula et al., 2012; Liebman et al., 2012). Mūsu pētījumā baltās, spīdīgās strēles tika novērotas 47 % AK pirms terapijas sākšanas. Iepriekš publicētos pētījumos šo struktūru sastopamība tika vērota 1,7 %–29,4 % AK (Balagula et al., 2012; Liebman et al., 2012).

Pie iemesliem, kāpēc baltās, spīdīgās strēles bija biežāk sastopamas mūsu pacientu grupā, salīdzinot ar citiem autoriem, var minēt pētījumā noteiktos iekļaušanas kritērijus. Konkrētāk, vērtējot AK dermatoskopiskās pazīmes, tika izslēgti veidojumi ar biezu zvīņu un hiperkeratozi, kas traucētu redzēt zem tām esošās struktūras, tostarp baltās, spīdīgās strēles. Būtiski ir ņemt vērā, ka zvīņas ir izplatīta AK pazīme un sastopama 79,4–85 % veidojumu (Lee et al., 2014; Zalaudek et al., 2006). Papildus tika izslēgti veidojumi, kuriem terapijas laikā attīstījās biezas kreveles vai erozijas. Vēl viens iemesls augstajai balto, spīdīgo strēļu izplatībai mūsu pētījumā varēja būt tas, ka pat atsevišķas strēles tika uzskatītas par pozitīvu pazīmi un izmantotā *FotoFinder Systems medicam 1000* ierīce sniedz 20 reižu lielu palielinājumu, tādējādi arī augstāku izšķirtspēju salīdzinājumā ar rokā turamām ierīcēm.

Mūsu pētījumā, lai gan izlase bija neliela, bija iespējams noteikt vairākus iespējamos scenārijus, kā baltās, spīdīgās strēles mainās terapijas ietekmē. Pirmkārt, lai gan veiksmīga terapija parasti ir saistīta ar dermatoskopisko pazīmju izzušanu, baltās, spīdīgās strēles var būt redzamas visos ārstēšanas posmos vai pat pirmo reizi parādīties mēnesi pēc terapijas bez citām dermatoskopiskām AK pazīmēm. Tā kā baltās, spīdīgās strēles nav obligāta AK pazīme, tad to

saglabāšanās vai parādīšanās nav pretnostatījums veiksmīgam terapijas iznākumam. Otrkārt, tāpat kā citas dermatoskopiskās struktūras, baltās, spīdīgās strēles var izzust terapijas radītā iekaisuma laikā un atjaunoties pēc tā. Šis pēdējais novērojums bija 13 % analizēto gadījumu, un, lai gan precīzs šādas parādības iemesls vēl nav skaidrs, tas liek domāt, ka baltās, spīdīgās strēles varētu būt neredzamas arī citos klīniski eritematozos bojājumos, ne tikai AK.

Turpinot izvērtēt AK dermatoskopisko struktūru mainību pirms un pēc terapijas, tika ziņots par interesantu klīnisko gadījumu, kurā, izārstējot rajonu ar klīniski un dermatoskopiski diagnosticētām AK, atsedzas dermatoskopiskas bazālo šūnu karcinomas struktūras. Šis klīniskais gadījums parāda, ka AK ārstēšana ļauj labāk klīniski un dermatoskopiski vizualizēt citus nepigmentētus ādas audzējus, tāpēc ir iespējama to agrāka diagnostika un ārstēšana. Tas savukārt uzskatāms par papildu ārstēšanas ieguvumu bez jau zināmā kosmētiskā ieguvuma un, iespējams, aizsardzības pret invazīvas plakanšūnu karcinomas attīstību.

Noslēdzošajā darba daļā tika pētīta smagu aplikācijas vietu reakciju izplatība, izmantojot lokālas AK terapijas metodes – dienas gaismas fotodinamisko terapiju, 5 % imihimoda krēmu, 3,75 % imihimoda krēmu, 0,015 % ingenola mebutāta gelu, 3 % diklofenaka gelu ar 2,5 % hialuronskābi, 0,5 % fluoruracila gelu un 0,5 % fluoruracila un 10 % salicilskābes kombinētu gelu. Lai to paveiktu, tika sistemātiski meklēti raksti *PubMed* meklētājā un apkopoti dati par smagu aplikācijas vietu reakciju sastopamību atkarībā no izmantotā medikamenta un par terapijas pārtraukšanu aplikācijas vietu reakciju dēļ. Netika iekļauti pētījumi un metodes, kurās nepieciešams īpašs aprīkojums, piemēram, konvencionālā fotodinamiskā terapija. Rezultātā tika apkopoti dati no 19 pētījumiem, un smagu aplikācijas vietu reakciju izplatība variēja no 0 % līdz 58,5 %. Visretāk smagas aplikācijas vietu reakcijas tiek novērotas, terapijā izmantojot dienas gaismas fotodinamisko terapiju, savukārt visbiežāk – terapijā ar imihimodu. Turklāt tikai shēmās ar imihimodu tika novērota arī sistēmisku simptomu attīstība. Papildus 14 rakstos tika minēta terapijas pārtraukšana aplikācijas vietas reakcijas dēļ. Visbiežāk – 4,9–13,6 % gadījumu – terapija tika pārtraukta, ārstējot ar 3 % diklofenaku, kas tiek nozīmēts lietošanai divas reizes dienā 3–6 mēnešus ilgi. Trijos pētījumos ar 5 % imihimodu terapijas pārtraukšana tika novērota līdz 3,2 % gadījumu, un tas tika skaidrots ar rūpīgu pacientu iepazīstināšanu ar aplikācijas vietu reakciju attīstību. Papildus tika noteikti ar terapiju nesaistīti riska faktori smagāku aplikācijas vietu reakciju attīstībai, un tie bija gaiša ādas krāsa (I–II fototips), sievietes dzimums, vecums līdz 70 gadiem, kā arī augstāka gaisa temperatūra, izmantojot dienas gaismas fotodinamisko terapiju (Fargnoli et al., 2015; Galvão et al., 2017; Ortega del Olmo and Salido-Vallejo, 2018; Ricci et al., 2016).

Secinājumi

1. Atbilstoši publicētajiem prospektīvajiem pētījumiem no ilgstošām un lielām vai saplūstošām aktīniskajām keratozēm visbiežāk attīstījās plakanšūnu karcinomas augsta riska populācijās. Tāpēc šīs pazīmes uzskatāmas par klīniskajiem riska faktoriem augstākai aktīnisko keratožu progresijai par plakanšūnu karcinomu.
2. Nepigmentētām aktīniskajām keratozēm sejas ādā raksturīgākās dermatoskopiskās pazīmes ir fona eritēma, baltas folikulu atveres, virsmas zvīņas, rozetes, smalki, lineāri, viļņaini asins kapilāri, mikroerozijas un saulē bojāta apkārtējā āda. Salīdzinājumam – sejas intraepidermālajām karcinomām ir papildu pazīmes, piemēram, sarkanas radiāli novietotas līnijas (zvaigžņu raksts), centrāli novietotas zvīņas vai keratīns, punktoti, glomerulāri vai cilpveida asins kapilāri un virspusēji izčūlojumi.
3. Histopatoloģiski redzamā amorfo masu klātbūtne, p53 krāsojuma intensitāte, Bcl-2 un CD31 ekspresija atšķiras klīniski veselā saules bojātā ādā, salīdzinot ar aktīnisko keratožu un intraepidermālo karcinomu ādas paraugiem.
4. Dermatoskopiski tipiskām aktīniskajām keratozēm histopatoloģijā biežāk ir redzamas amorfas masas un ir zemāka CD31 marķiera ekspresija. Savukārt balto folikulu zudums ir saistīts ar palielinātu Bcl-2 subepidermālo ekspresiju.
5. Baltās, spīdīgās strēles ir mainīgas dermatoskopiskās struktūras, kuras var izzust terapijas radītā iekaisuma fāzē un pēc terapijas, kā arī var parādīties pirmo reizi pēc terapijas. Novērtējot balto, spīdīgo strēļu klātbūtni, ir būtiski ņemt vērā to dinamisko dabu.
6. Aktīnisko keratožu ārstēšana sniedz iespēju noteikt pret terapiju rezistentus veidojumus, kas pēc terapijas saglabā savas dermatoskopiskās īpašības un ļauj labāk klīniski un dermatoskopiski vizualizēt citus veidojumus, tādējādi vēl vairāk veicinot agrīnu ādas vēža diagnostiku.
7. Smagas aplikācijas vietu reakcijas ir ļoti biežas aktīnisko keratožu lokālas terapijas laikā, īpaši lokāli lietojot imihimodu. Vienīgā terapeitiskā metode, kam smagu lokālu aplikācijas vietas reakciju izplatība ir zema, ir dienas gaismas fotodinamiskā terapija. Ārstēšanas pārtraukšana lokālu reakciju dēļ ir izplatīta, lai gan visbiežāk ārstēšana tiek pārtraukta, ārstējot ar ilgākajām ārstēšanas shēmām, piemēram, ar diklofenaku, nevis ārstējot ar medikamentiem, kas rada izteiktākās aplikācijas vietu reakcijas.

Priekšlikumi

1. Ja nav iespējama visu aktīnisko keratožu vienlaicīga terapija, tad ilgstošas un lielas vai saplūstošas aktīniskās keratozes būtu jāārstē primāri, jo tām ir augstāks risks progresēt par plakanšūnu karcinomām.
2. Dermatoskopija ir ieteicama diagnostikas metode visām aktīniskajām keratozēm pirms un pēc terapijas.
3. Veikt diagnostisku biopsiju jebkurai aktīniskajai keratozei, kurai dermatoskopijā ir redzama kāda atipiska pazīme, lai izslēgtu intraepidermālu karcinomu vai agrīnu invazīvu plakanšūnu karcinomu.
4. Visiem pacientiem ir rūpīgi jāizskaidro aktīnisko keratožu lokālās terapijas ieguvums un paredzamās reakcijas aplikācijas vietās, jo tas samazina ārstēšanas pārtraukšanas biežumu.
5. Veikt papildu pētījumus par dienas gaismas fotodinamiskās terapijas iespējām aktīnisko keratožu ārstēšanā Latvijā, jo šīs terapijas gadījumā medikaments ir jāuzklāj vienreiz un tā ir saistīta ar visretāko smagu aplikācijas vietu reakciju attīstību.

Publikāciju, ziņojumu un patentu saraksts par promocijas darba tēmu

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Systematic Review

Clinical Characteristics of Actinic Keratosis Associated with the Risk of Progression to Invasive Squamous Cell Carcinoma: A Systematic Review

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Abstract: Background: Actinic keratosis (AK) is one of the most common lesions on chronically sun-damaged skin that has the risk of progression to invasive squamous cell carcinoma (SCC). With the possibilities of using digital technologies for following-up skin lesions and their increased use in the past few decades, our objective was to update the review by Quaedvlieg et al., 2006, and to review prospective studies from 2005 onwards to identify the clinical characteristics of AK that later progressed to SCC. Methods: The PubMed, Scopus, and ScienceDirect databases were searched for relevant articles. The search had the following criteria: English language, human subjects and year from 2005 onwards. The study protocol was registered in the Prospero database with the record number CRD42020200429 and followed the PRISMA guidelines. The risk-of-bias assessment was performed using the QUIPS tool. Results: From the 5361 studies screened, 105 reports were evaluated for eligibility, and 2 articles with 621 patients were included. The main AK types associated with the development of SCC were found to be baseline AK, also known as a long-standing AK, and merging AK, also called an "AK patch".

Keywords: AK patch; merging AK; baseline AK; keratinocyte cancer; progression

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1. Introduction

Squamous cell carcinoma (SCC) is the second most common skin cancer in humans [1] and has several distinct in situ and invasive types. Actinic keratosis (AK) is a well-recognized precursor of cutaneous SCC that is caused by the long-term exposure of the skin to ultraviolet radiation [2] and is one of the most common reasons for dermatology office visits [3]. The usual clinical presentation of AK is a scaly erythematous macule or patch on a sun-exposed area. In ambiguous cases, histopathological confirmation is needed to differentiate AK from SCC. The progression from AK to invasive SCC has been previously described [4–6], and the risk of progression is estimated to be between 0.025% and 20% for each individual lesion [2,6,7]. A study has shown that the process of progression takes approximately 2 years for lesions that warrant histological confirmation [6].

Although some authors state that it is not possible to predict which AKs will progress to invasive SCC [8] and that these lesions are more a general marker of the risk of SCC than true precursors [9], a study by Quaedvlieg et al., published in 2006 identified several clinical findings associated with the malignant progression of AK and invented the acronym IDRBEU. In the acronym, "I" stands for inflammation/induration; "D", for a diameter > 1 cm; "R", for rapid enlargement; "B", for bleeding; "E", for erythema; and "U", for ulceration. Additional minor clinical criteria identified in their study were pain, palpability, hyperkeratosis, pruritus, and pigmentation [10].

As new studies and new technologies have been implemented in dermatology to perform long-term follow-ups of separate lesions, we believe that new evidence of the clinical features of AK progressing to SCC should be available. Therefore, we decided to update the review by Quaedvlieg et al. [10].

2. Materials and Methods

The study's protocol was registered in the Prospero database with the record number CRD42020200429 [11] and followed the PRISMA guidelines for reporting systematic reviews [12].

The PubMed, Scopus, and ScienceDirect databases were searched for relevant studies on 28 July 2020. The search was restricted to years from 2005 onward. The search was limited to papers written in the English language and studies involving human subjects. The following inclusion criteria were used: patients with diagnoses of AK (P), information on follow-up was provided or a longitudinal assessment was performed (I), and the development of SCC (O) was recorded in a prospective manner (S). The exclusion criteria were a lack of data on previous AK (P); no clinical characteristics of AK being mentioned (P); treated AK (I); no prospective data being available (I); no development of SCC being mentioned (O); no data regarding SCC development from AK being presented (O); review articles (S); and articles with follow-up periods less than 3 months (S). The search strings were composed in collaboration with the Riga Stradiņš University Library and can be found in the registered protocol [11]. The search results were extracted and uploaded to the Covidence system for the removal of duplicates and the selection of relevant titles and abstracts. There were minor author changes from the registered protocol. Two authors (A.B. and L.K.Y.) were assigned, and they independently performed the title and abstract selection. In the case that an abstract was unavailable, the full text was screened at the initial stage. All the discrepancies were resolved in discussions with I.C. after the manual extraction of conflicting articles. The full texts of the selected articles were reviewed independently by A.B. and L.A.P.; all the discrepancies were resolved in discussions with L.K.Y. and I.C. The reference lists of eligible studies were manually screened for additional relevant articles. The search was rerun from 2 to 3 February 2022 to include the latest articles. The risk-of-bias assessment for the selected articles was performed using the QUIPS tool for assessing the risk of bias in prognostic factor studies by A.B. and L.K.Y. All the disagreements were resolved in discussions with I.C.

3. Results

The PRISMA flow diagram of the article selection process is depicted in Figure 1. In total, 5361 titles and abstracts were screened, 105 articles were evaluated for eligibility, and 2 studies with 621 patients were included [13,14]. Both of the included studies had a low risk of bias, as evaluated with the QUIPS tool (Figure 2) for assessing the risk of bias in prognostic factor studies. Both of the studies had patients at high risk for the development of SCC.

First, a study by Criscione et al. [13] longitudinally examined a group of veterans from the Department of Veterans Affairs Topical Tretinoin Chemoprevention Trial. The study cohort consisted of 169 participants who had been diagnosed with more than two keratinocyte carcinomas in the 5 years prior to enrollment. The participants were examined at approximately 6-month intervals for a mean of 7 examinations (range: 2–16 examinations). During each examination, high-resolution standardized digital photographs were taken. In total, 187 primary SCCs on the face or ears developed in the study. Of those, 65% (91 invasive and 31 in situ SCCs) were diagnosed from previously documented AK. The main type of AK associated with the development of SCC was baseline AK, which showed an increased risk of progression to primary SCC (invasive or in situ; $p = 0.02$) but not to primary invasive SCC ($p = 0.17$). The risk of progression from baseline AK to in situ or invasive SCC was 3.13% at 3 years and 4.03% at 5 years.

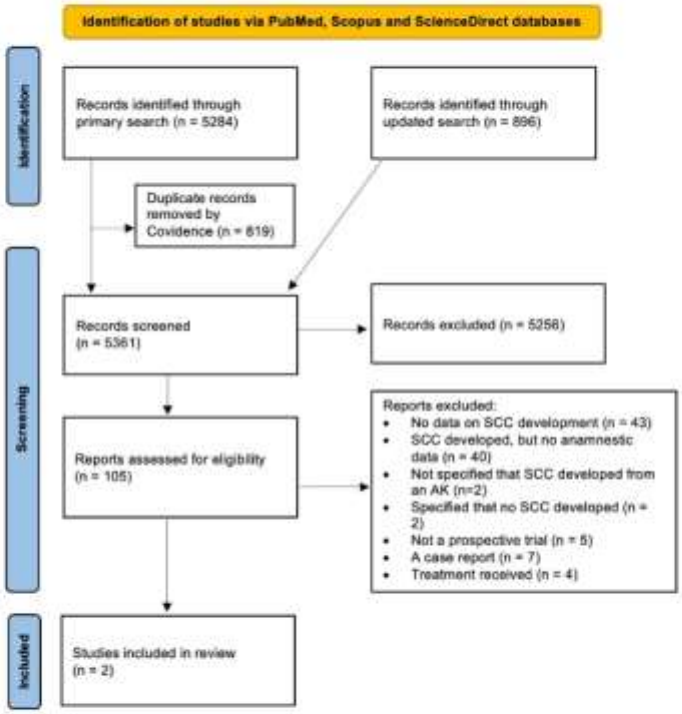

Figure 1. PRISMA flow diagram of the article selection process. Two studies met the inclusion criteria. AK—actinic keratosis. SCC—squamous cell carcinoma.

Figure 2. Evaluation of risk of bias using the QUIPS tool. Both studies were considered to have an overall low risk of bias. QUIPS, Quality in Prognosis Studies [13,14].

In the second study by Wallingford et al. [14], a dermatologist examined and performed a routine follow-up from May 2010 to October 2011 for a representative cohort of 452 white renal transplant recipients (RTRs). In total, 130 (29%) of the participants had AK at the time of examination and were examined by a dermatologist every 3–4 months [15]. The authors defined merging AK, namely, skin areas greater than 1 cm^2 with confluent erythema and scaling, as actinic field changes. During the study period, 20 (4%) RTRs were diagnosed with SCC. Of those, 11 (55%) developed the malignancy directly in an area of field change. One RTR developed two SCCs in areas of field change at two different sites. The field change increased the risk of SCC by 93-fold.

In both of the included studies, the patients received no specific AK treatment except sunscreen and tretinoin, which had no effect on SCC or AK reduction.

4. Discussion

This systematic review identified baseline or pre-existing AKs and large AKs that exceed 1 cm^2 in diameter as the main clinical features of AK that are associated with the development of SCC in prospective longitudinal studies.

The development of SCCs from baseline or pre-existing AKs is in agreement with the well-established mode of progression and has been supported by clinical and histological studies [5,6]. Clinically, this can be translated as meaning that the lesions initially diagnosed in a patient are more likely to develop into SCC than are those that develop during a follow-up. Similarly, this is in accordance with the time required for the development of SCC: the longer the lesion is present, the higher the probability of SCC development [13].

The development of SCCs from areas of confluent AKs or large AKs that exceed 1 cm^2 in area agrees with the findings from a previous study by Quaedvlieg et al. [10], who highlighted a diameter of more than 1 cm as a risk factor for the development of SCC. In some studies, large AKs exceeding 1 cm^2 have been called AK patches and have similarly been associated with the development of SCC. For example, in two studies by Jiyad et al. [16,17], signs predicting the development of SCC in a certain area were AK patches, the number of AK patches, three or more AKs at a single anatomical site, and the percentage of the area involving AK > 25%. Jiyad et al., defined an area by dividing the face into five areas, in addition to each ear as a separate area. Both studies were excluded from our review, as there was no information indicating that SCCs had developed from the AK patches themselves. Furthermore, large areas of confluent AKs have also been linked to the more aggressive behavior of SCCs [18].

Both the included studies had patients at high risk for SCC development, namely, patients with previous keratinocyte carcinomas and renal transplant recipients. It is known that immunosuppressed patients have a higher prevalence of AK and a higher risk of the progression of these lesions to SCC. This among other reasons can be attributed to the use of immunosuppressive medications that cause direct damage to the DNA when the patient is exposed to UVA radiation; in addition, these medications are photosensitizing and also affect the correction pathways of pre-oncogenic mutations [19].

In the previous study by Quaedvlieg et al. [10], the identified clinical features were derived from four literature reviews and a single prospective study by Suchniak et al. [20]. In the latter, it was found that clinical hyperkeratotic AKs less than 1 cm in diameter on the hands, wrists, and forearms of white patients who have had severe actinic damage are often invasive SCCs (in 36% of cases). Therefore, the only clinical feature included in the risk factors associated with a malignant progression of AK into SCC from prospective trials in the study by Quaedvlieg et al. [10] was hyperkeratosis. Our study did not find hyperkeratosis as a feature associated with SCC development in prospective longitudinal studies. Moreover, studies have shown that only 14% of hyperkeratotic lesions correspond to grade III AKs on histopathology with atypical keratinocytes extending to more than two-thirds of the full thickness of the epidermis [21].

In the final step of the screening, five case reports were reviewed, from which additional information was obtained. In these reports, the mean age of the patients was 84 years (range: 73–101), and in all cases, the SCC developed from an AK on the face. In two cases where information on the size of the AK was available before the development of SCC, it was at least 1.6 cm in diameter [22,23]. In four cases, pre-existing AK was present for several years [23–26], and in four cases, a recent rapid growth was observed [22,24–26]. In only one of the reported cases, where SCC developed from a previous AK, was the patient specified to have no other AK lesions [24].

The strengths of this study include the comprehensive data search, the registered protocol, the adherence to reporting guidelines, and the large amount of literature reviewed.

However, there are several limitations of this study. First, only a low number of studies were included. Second, we were unable to identify studies on low-risk patients. The probable reasons include a lower risk of SCC development in immunocompetent patients, complicated study designs, the high costs of such studies, the high numbers of AKs a single individual can have, and the fact that digital follow-up is more commonly used for melanocytic skin lesions. In addition, only databases, but no other sources, were searched.

5. Conclusions

In comparison to previous reviews, longitudinally assessed features of AK progression to invasive SCC have been summarized in this article. We conclude that long-standing and large or merging actinic keratoses, sometimes called AK patches, are the most important risk factors for the development of SCC in high-risk populations.

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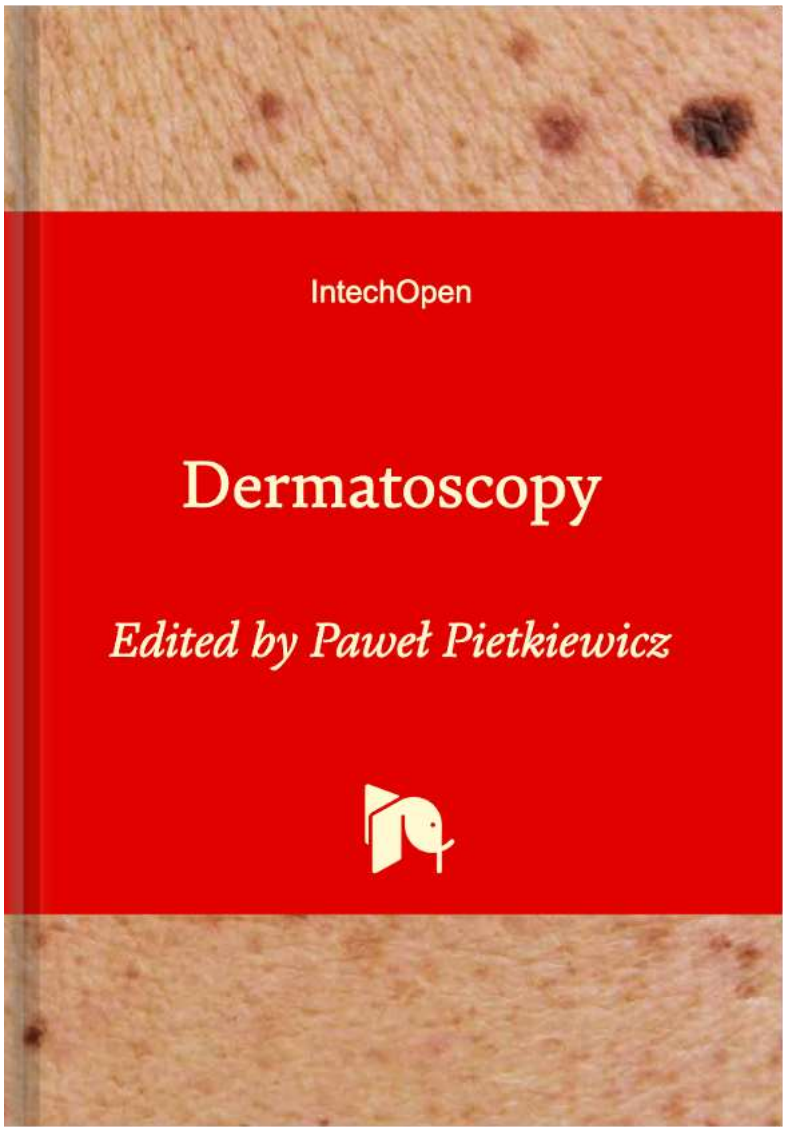

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Chapter

Dermatoscopy of Facial Non-Pigmented Actinic Keratosis and Intraepidermal Carcinoma

Alise Balcere

Abstract

Dermatoscopy improves the diagnostic accuracy of non-pigmented facial lesions, including actinic keratosis (AK) and intraepidermal carcinoma (IEC) and helps to differentiate them from common invasive malignancies such as basal cell carcinoma and invasive squamous cell carcinoma. The most common dermatoscopic features characterizing AK are background erythema/erythematous pseudonetwork, white follicular openings/targetoid hair follicles, surface scales, rosettes, fine, linear, wavy vessels, microerosions and sun-damaged surrounding skin. In comparison, the most common dermatoscopic features of IEC are background erythema, red starburst pattern, surface scale, dotted/glomerular vessels, hairpin vessels, microerosions/ulcerations and targetoid hair follicles. The practice of recognizing these features in dermatoscopic images is a useful tool in the armamentarium of a clinician examining skin lesions.

Keywords: actinic keratosis, erythematous facial lesions, squamous cell carcinoma in situ, bowenoid actinic keratosis

1. Introduction

Actinic keratosis (AK) and other forms of squamous cell carcinoma (SCC) in situ are among the most common lesions in dermatological practice and are primarily the result of cumulative UV damage. The clinical relevance of accurate diagnosis relies on several factors. Firstly, misdiagnosing an inflammatory disease as an AK would lead to unnecessary and possibly harmful usage of destructive therapies on benign lesions. Secondly, AK is commonly a lesion in a field of sun-damaged skin, and among other lesions associated with chronic sun damage, some small clinically indistinguishable carcinomas may rest. Moreover, AK, although a common lesion, might progress to invasive SCC with gradual changes that can be visualized under a dermatoscope [1]. Furthermore, studies have shown that most SCCs arise from or in close proximity to AK and that dermatoscopy aids in differentiation between AK and SCC [2, 3]. Therefore, dermatoscopy is a useful tool for a clinician examining non-pigmented facial lesions allowing to differentiate between them.

Several forms of in situ SCC that are united by atypical keratinocytes in the epidermis but vary clinically, dermatoscopically, and histopathologically have been recognized [4]. Actinic keratosis (AK) and intraepidermal carcinoma (IEC) are the two main types of SCC in situ affecting facial skin. Much less common forms

include arsenical keratosis, radiation keratosis (caused by ionizing radiation), and hydrocarbon keratosis, in which dermatoscopic differences have not been described [5]. The following chapter will provide an overview of the clinical and dermatoscopic features that characterize different forms of AK and IEC of the face, including the dermatoscopic progression model from AK to invasive SCC.

2. Definition of actinic keratosis and intraepidermal carcinoma

The differentiation between AK and IEC relies on their histopathologic characteristics.

AK is also called solar or senile keratosis, SCC in situ AK-type, or keratinocytic intraepidermal neoplasia and represents a common lesion on chronically sun-damaged skin of fair skinned individuals. Histopathologically, AK presents as atypia of basal keratinocytes with loss of polarization, crowding, and overlapping that can extend up to near full thickness atypia in advanced lesions [5–8].

IEC is an intraepithelial SCC exhibiting full-thickness cellular dysplasia [9]. However, other synonyms employed for extragenital full-thickness intraepidermal carcinoma are Bowen's disease, in situ SCC, cutaneous SCC in situ, and intraepithelial SCC [1]. It is noteworthy that in comparison with other types of SCC in situ, Bowen's disease has been defined as SCC in situ arising on sun-protected skin, without field damage and possibly without association with HPV, although previously suggested otherwise [10–12]. For the consistency of this chapter, the term "*intraepidermal carcinoma*" will be used to describe facial intraepithelial SCC exhibiting full-thickness cellular dysplasia.

3. Diagnosing AK and IEC

Actinic keratosis in the majority of cases can be diagnosed clinically. Nevertheless, the clinical description of an erythematous macule or patch with a superficial scale may correspond to many other skin lesions and dermatoses. Studies [13, 14] examining the diagnostic precision of clinically diagnosed AK have reported misdiagnosis rates of approximately 10%. The main biopsy diagnoses in cases of misdiagnosis were SCC in situ, SCC with superficial invasion, seborrheic keratosis, basal cell carcinoma, and other benign skin lesions and dermatoses such as subacute spongiotic dermatitis, rosacea, solar elastosis, scars and verrucae plana. Pivotal differential diagnosis of AK is invasive SCC that can mimic AK if presenting as an erythematous macule. It has been shown that 1.5% of clinically diagnosed AK lesions identified by board-certified dermatologist were SCCs with superficial invasion on histologic assessment [13]. In comparison, dermatoscopy improves the diagnostic accuracy of both AK and SCC. A recent systematic review and study by Huerta-Brogeras *et al*. showed sensitivity up to 98.7% and specificity up to 95% if AK is diagnosed with dermatoscopy [15, 16].

Diagnosis of IEC is based on clinical, dermatoscopic, and histopathologic features.

3.1 Clinical features of AK and IEC

The most frequent presentation of both AK and IEC is a variably erythematous scaly patch or slightly elevated plaque [17]. AK is either single or multiple, while IEC is usually a single lesion. In comparison with AK, IEC is often an indurated

lesion on palpation. Both lesions are asymptomatic in most of the cases, although some patients experience discomfort, such as burning, pain, bleeding, and pruritus [6]. It has been noted that pain can be equally present in both AK and IEC, but is more common in invasive SCC [18].

A broad and useful tool for clinical description of the thickness of AK is a classification by Olsen *et al*. [19]. In this classification:

- Grade 1 AKs are mild - slightly palpable, better felt than seen.
- Grade 2 AKs are moderately thick that are easily seen and felt.
- Grade 3 AKs are severe - very thick, hyperkeratotic, and obvious AK.

However, this clinical classification cannot reliably predict the histological grade proposed by Roewert-Huber *et al*. that could justify the classical progression model of AK to invasive SCC through clinical thickening and histopathological upward extension of atypical keratinocytes before invasion. It has been shown that only 26% of Olsen grade 1 lesions were grade 1 on histopathology with atypical keratinocytes in the basal and suprabasal layers of the epidermis, 75% of Olsen grade 2 lesions were grade II on histopathology with atypical keratinocytes extending to the lower two-thirds of the epidermis and only 14% of Olsen grade 3 lesions had corresponding grade III on histopathology with atypical keratinocytes extending to more than two thirds of the full thickness of the epidermis [8, 20].

3.2 Dermatoscopic features of AK and IEC

For the description of dermatoscopic features of AK and IEC, both metaphoric and descriptive language can be used. Definitions of the main metaphoric and descriptive terms are given in **Table 1**.

Main dermatoscopic features of AK are depicted in **Table 2**. Main dermatoscopic features of IEC are depicted in **Table 3**.

Metaphoric/descriptive terms	Definition
Red pseudonetwork	Marked pink-to-red background erythema surrounding accentuated hair follicles
Red starburst pattern	Radially arranged structureless red lines or hairpin vessels that surround a yellow to white structureless scaly center and that resemble an overall starburst appearance
Rosettes	Four bright white dots or clods arranged together as a square (or 4-leaf clover)
Shiny white streaks	Short discrete white lines oriented parallel and orthogonal (perpendicular) to each other seen only under polarized dermatoscopy
Strawberry pattern	Red pseudonetwork in combination with targetoid hair follicles
Targetoid hair follicle	Yellowish keratotic plug within a prominent hair follicle opening surrounded by a white halo
White circles	Bright white circles surrounding an orange/yellow keratin plug

Table 1.
Standardized terms of common dermatoscopic features for AK and IEC [18, 21–23].

Classic AK	**Most common dermatoscopic findings**	
	• Background erythema/erythematous pseudonetwork	**Strawberry pattern**
	• Follicular openings/ targetoid hair follicles	
	• Surface scales ○ Yellow-white opaque scales ○ Diffuse/discrete scales • Rosettes • Fine, linear, wavy vessels • Microerosions • Sun damaged surrounding skin	
	Less common, but possible findings	**Structure is more characteristic to**
	• Central scale	IEC, SCC, KA
	• Dotted/glomerular vessels	IEC
	• White structureless areas (common in Korean patients)	SCC, KA
	A rare finding	**Structure is more characteristic to**
	• Central ulceration	SCC, KA
	• Linear-irregular vessels	KA, SCC
	• Hairpin vessels	SCC, KA, IEC
	• Red starburst pattern	IEC, SCC
	• Shiny white streaks	Dermatofibroma, scar, BCC
Bowenoid AK	**Most common dermatoscopic findings**	
	• Glomerular vessels regularly distributed	
	• Surface scale	
Hyperkeratotic AK	**Most common dermatoscopic findings**	
	• Marked hyperkeratosis seen as white-yellow structureless areas preventing visualization of underlying structures	

Table 2.
Dermatoscopic features of AK categorized in three groups according to their prevalence. The most common dermatoscopic findings – Features present in almost all to the majority of AKs. Less common, but possible findings – Present in some AKs, although more common and characteristic for other lesions. A rare finding – Sometimes present in AK, but a differential diagnosis is much more likely. Abbreviations: AK – Actinic keratosis; IEC – Intraepidermal carcinoma; KA - Keratoacanthoma; SCC – Squamous cell carcinoma; BCC – Basal cell carcinoma [1, 6, 15, 22–26].

3.2.1 Characteristics of specific features

3.2.1.1 Erythematous pseudonetwork

Erythematous pseudonetwork can be defined as a marked pink-to-red background erythema formed by fine wavy telangiectatic vessels surrounding accentuated hair follicles [23]. It is one of the most common and characteristic findings of AK.

IEC	Most common dermatoscopic findings	
	• Background erythema	
	• Red starburst pattern	
	• Surface scales	
	○ Yellow-white opaque scales	
	○ Central scale	
	○ Diffuse/discrete scales	
	• Dotted/glomerular vessels	
	• Hairpin vessels	
	• Microerosions/ulcerations	
	• Targetoid hair follicles	
	Less common, but possible findings	**Structure is more characteristic to**
	• Rosettes	
	• Central keratin mass	KA, SCC
	• Red pseudonetwork	AK
	A rare finding	**Structure is more characteristic to**
	• White structureless areas	KA, SCC
	• Linear-irregular vessels	KA, SCC
	• Central ulceration	BCC, SCC, KA

Table 3.
Dermatoscopic features of IEC categorized in three groups according to their prevalence. The most common dermatoscopic findings – Features present in almost all to majority of IECs. Less common, but possible findings – Present in some IECs, although more common and characteristic for other lesions. A rare finding – Sometimes present in IEC, but a differential diagnosis is much more likely. Abbreviations: AK – Actinic keratosis; IEC – Intraepidermal carcinoma; KA - Keratoacanthoma; SCC – Squamous cell carcinoma; BCC – Basal cell carcinoma [1].

3.2.1.2 Targetoid hair follicles

Targetoid hair follicles are formed by yellowish keratotic plugs within the hair follicles and surrounded by a whitish halo. This feature is particularly common for AK on the nose and hyperkeratotic AK [23].

3.2.1.3 Strawberry pattern

Strawberry pattern (**Figure 1**) is a composite appearance of reddish pseudonetwork and hair follicles. This pattern is present in up to 95% of AK [23].

3.2.1.4 Surface scales

Scales are one of the most common features of AK and correlate with hyperkeratosis and parakeratosis on histopathology [21]. The distribution is usually diffuse throughout the lesion, although some lesions can be partly scaly (**Figure 1**) and a central scale is common for hyperkeratotic lesions. The color of the scales varies from white to yellow and an accumulation of exogenous pigment has been reported [27].

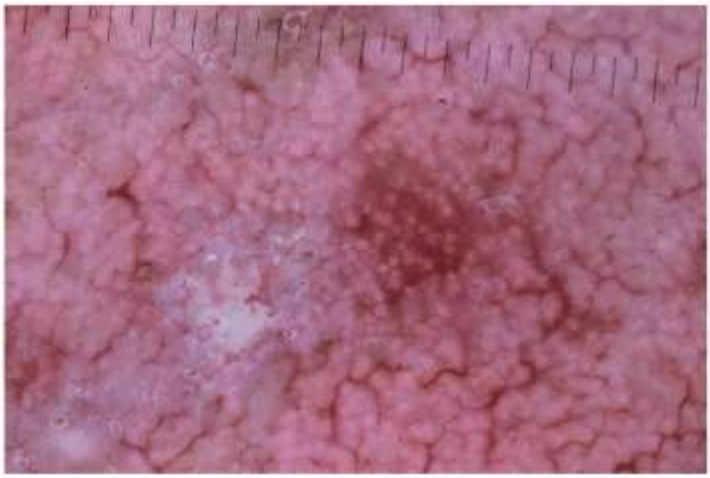

Figure 1.
Dermatoscopic image of an AK. White scales limiting visualization of the underlying structures are seen on the left side of the picture, while a typical strawberry pattern with erythematous pseudonetwork and targetoid hair follicles are seen on the right side.

3.2.1.5 Rosettes

Rosettes are also named 4-dotted-clods in descriptive terminology. Rosettes are a clue for keratinizing neoplasms, although they can also be observed in several other conditions including basal cell carcinoma, melanoma, melanocytic nevus, dermatofibroma, scar, molluscum contagiosum, actinically damaged skin and cicatricial alopecia of lichen planopilaris [28]. The dermatopathological correlate of 4-dotted-clods in AK is horizontally arranged alternating hyperkeratotic and parakeratotic corneal layers in the follicular infundibula associated with mild peri-follicular fibrosis [28]. It has also been proposed that smaller 4-dotted-clods are caused by the concentric horn in the follicle at the infundibular level, whereas larger ones are caused by concentric fibrosis around the follicle [29].

3.2.1.6 Fine, linear, wavy vessels

Focused linear wavy vessels surrounding the hair follicles was found in more than 80% of facial AKs in a study by Zalaudek *et al*. These peculiar linear, wavy vessels of facial AK clearly differ in morphology from the arborizing vessels of vessels of nodular basal, short fine telangiectatic vessels of superficial basal cell carcinoma, and regular hairpin vessels that are characteristic of seborrhoeic keratosis. Furthermore, wavy vessels typically encircle the hair follicles as single and uniform units, which contrasts with the irregularly sized and distributed linear irregular vessels that can be seen in amelanotic/hypomelanotic melanoma, areas of regression in melanoma, or invasive SCC [23].

3.2.1.7 Microerosions

Microerosions are small erosions on the surface of the lesion seen under a dermatoscope. Microerosions are twice as common in IEC in comparison with AK, but are also a common feature of superficial basal cell carcinoma [1].

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3.2.1.8 Shiny white streaks

Shiny white streaks (SWS) are also known as chrysalis or crystalline structures by their metaphoric terms. Dermatoscopically, SWS are only visible in a polarized light dermatoscopy as white, perpendicular, few millimeters long lines. Histopathologically, SWS are caused by polarization of thickened hyaline fibrous bundles and therefore considered as a dermatoscopic sign of dermal fibrosis. Shiny white streaks have been reported in a variety of skin lesions, mainly dermatofibromas, scars, basal cell carcinomas, lichen planus like keratosis, invasive melanoma, melanoma metastasis and sometimes even solar lentigo and intradermal nevus. In addition, it has been reported that SWS might be less common in inflamed lesions [22, 25, 29–31].

3.2.1.9 Sun damaged surrounding skin

The importance of recognizing the features of the surrounding skin is based on several factors. First of all, AK quite commonly has a confluent solar lentigo on the border. Secondly, it has been hypothesized that humans focus on the lesion and not on the surrounding skin and therefore are outperformed by artificial intelligence in the precision of AK diagnosis. Moreover, teaching medical students to pay attention to chronic sun damage in the background improved the frequency of correct diagnoses of pigmented actinic keratoses from 32.5% to 47.3% [26]. In addition, lesions arising in field cancerization have a higher potential for malignant progression. The latter has been recognized in a new nomenclature of keratinocyte cancers by Conforti *et al.* According to the authors, all keratinocyte cancers should be classified in two groups - 'cSCC+field' for keratinocyte cancers arising in the presence of AK within the field of cancerization and 'cSCC-field' for keratinocyte cancers arising in the absence of AK or field cancerization [32].

3.2.1.10 Red starburst pattern

Red starburst pattern can be defined as radially arranged structureless red lines or hairpin vessels that surround a yellow to white structureless scaly center and that resemble an overall starburst appearance (**Figure 2**). Red starburst pattern is equally common in IEC and invasive SCC, and less common in AK [1].

3.2.1.11 Dotted/glomerular vessels

Dotted vessels are tiny red dots densely aligned next to each other [1]. Glomerular vessels are larger-caliber reddish dots formed by tortuous capillaries curled up into a ball and resembling the glomerular apparatus of the kidneys. Glomerular vessels are specific for Bowen's disease, if located in clusters and bowenoid AK, if distributed regularly. Glomerular vessels can also be present in stasis dermatitis, psoriasis, irritated seborrheic keratosis, superficial basal cell carcinoma and melanoma [33–35]. The combination of clustered dotted/glomerular vessels and hyperkeratosis has been previously shown to achieve a 98% diagnostic probability for IEC [1, 35].

3.2.1.12 Hairpin vessels

Hairpin vessels are vessels that double back on themselves and are seen as loops when they are oblique to the surface of the lesion. Hairpin vessels are a common feature of keratinizing tumors and are a hallmark of seborrheic keratosis in which

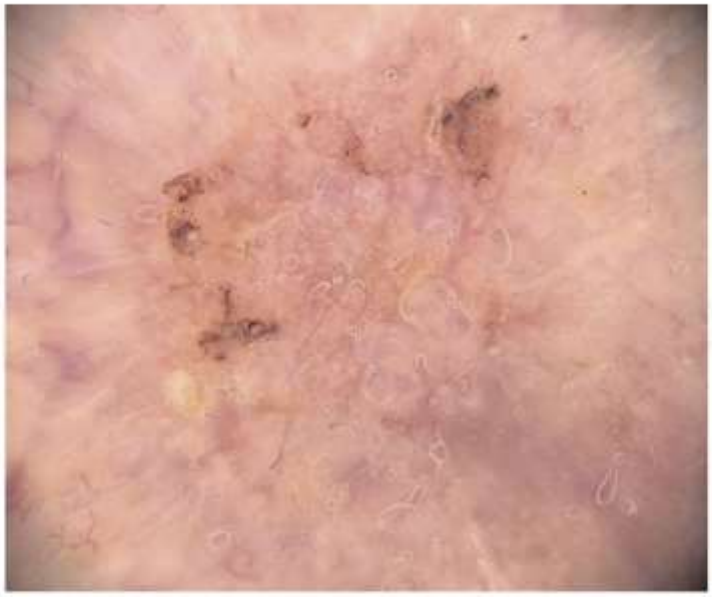

Figure 2.
Dermatoscopic image of IEC presenting with red starburst appearance formed by red and white radially arranged lines and central pink structureless clods, yellow scales, and hemorrhagic crusts.

they are usually regularly distributed and surrounded by a white halo. Hairpin vessels are a rare but possible finding in AK and a common finding in IEC and SCC. Hairpin vessels are associated with progression of IEC to invasive SCC and clinically thicker lesions. Positive predictive value of hairpin vessels for seborrheic keratosis is 70%, contrasting with only 13.3% for squamous cell carcinoma [1, 33].

3.2.2 Variants of AK

Apart from classical AK, other forms categorized histopathologically are hypertrophic, atrophic, bowenoid, acantholytic, pigmented, lichenoid, and proliferative variants, although in this grading system overlap of histologic subtypes may occur in a single lesion [36].

Atrophic AK. In this form, the lesion has an atrophic epidermis on histopathology [5]. According to one study, atrophic type AK more commonly presents with red pseudonetwork [37].

Bowenoid AK has a characteristic dermatoscopic feature of glomerular vessels regularly distributed along the lesion (**Figures 3** and **4**), thus differentiating it from Bowen's disease, whose vessels are irregularly distributed and grouped [6].

Hyperkeratotic AK presents with a nonspecific dermatoscopic pattern due to hyperkeratosis, which prevents visualization of the underlying structures [6]. In addition, it has been shown that the surface keratin of AK can accumulate exogenous pigmentation, particularly from broad spectrum sunscreens containing titanium dioxide. Such a specific feature of bright arctic-blue or greenish-blue color of AK on polarized light dermatoscopy has been described and named an "iceberg sign" [27].

Lichenoid AK clinically presents with pronounced erythema around the base of the lesion secondary to an underlying lichenoid infiltrate on histopathology [5]. Dermatoscopically, lichenoid AK might also present with a more intense erythematous background.

Dermatoscopy of Facial Non-Pigmented Actinic Keratosis and Intraepidermal Carcinoma
DOI: http://dx.doi.org/10.5772/intechopen.98875

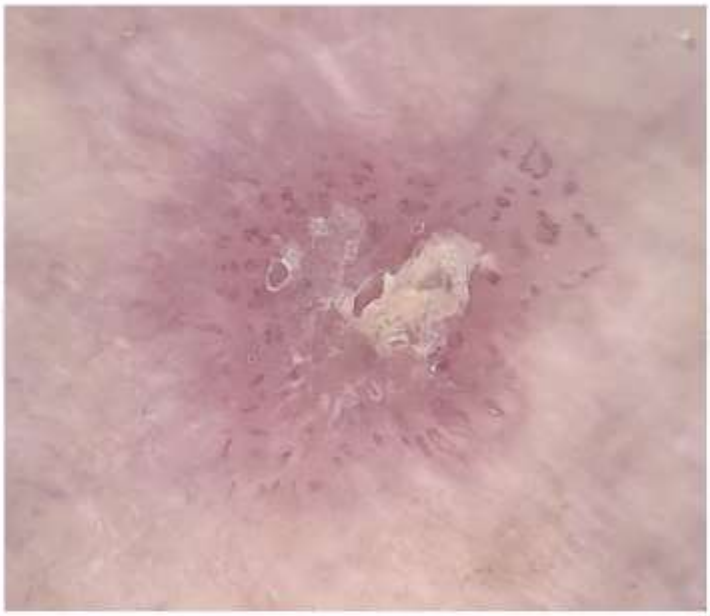

Figure 3.
Dermatoscopic image of bowenoid AK. Dermatoscopically regularly distributed glomerular (upper left) and hairpin (right and lower part) vessels in addition to a central white scale are seen.

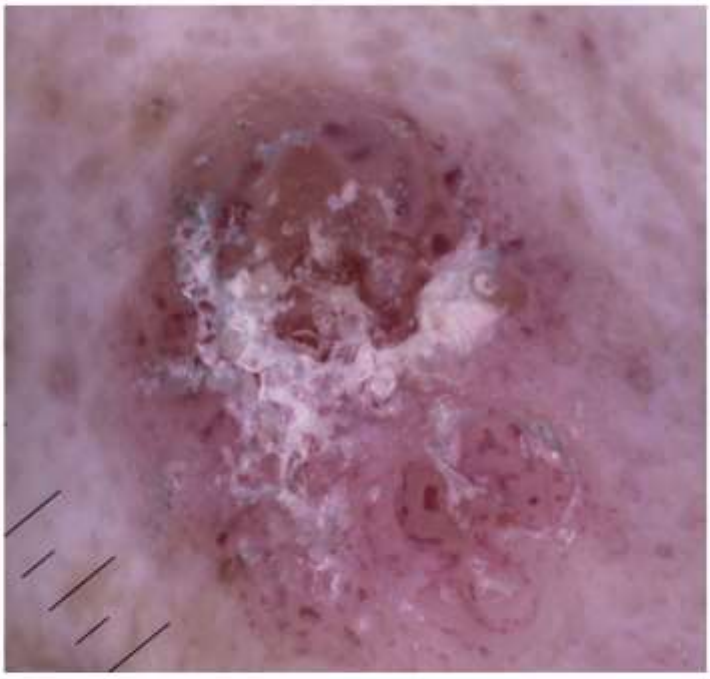

Figure 4.
Dermatoscopic image of bowenoid AK. Dermatoscopically regularly distributed dotted and glomerular vessels, white surface scales, yellow clods corresponding to hyperkeratosis (upper part), and few milia like cysts (lower left fragment) are seen.

3.2.3 Dermatoscopic–histopathologic correlations of AK

Skilled observers can predict the histologic grade of AK with dermatoscopy, although in consensus with clinical features some studies do not find such correlations [37, 38]. The following dermatoscopic–histopathologic correlations have been previously proposed:

- Grade 1 AK on dermatoscopy is typified by a red pseudonetwork and discrete white scales; this pattern correlates with grade I on histopathology where the keratinocytic atypia is mild and limited to the basal and suprabasal layers of the epidermis.
- Grade 2 AK is dermatoscopically characterized by an erythematous background intermingled with white to yellow, keratotic, and enlarged follicular openings. This described pattern in dermatoscopy resembles the surface of a strawberry, therefore was originally termed a strawberry pattern. In grade 2 AK, the histopathological changes are diffuse, with the lower two-thirds of the epidermis involved by atypical keratinocytes with alternating orthokeratosis and parakeratosis on the surface.
- Grade 3 AKs dermatoscopically exhibit either enlarged follicular openings filled with keratotic plugs over a scaly and white-yellow-appearing background or marked hyperkeratosis seen as white-yellow structureless areas. This grade on dermatoscopy corresponds to full-thickness atypia with increased mitotic activity and hyperkeratosis/parakeratosis [39].

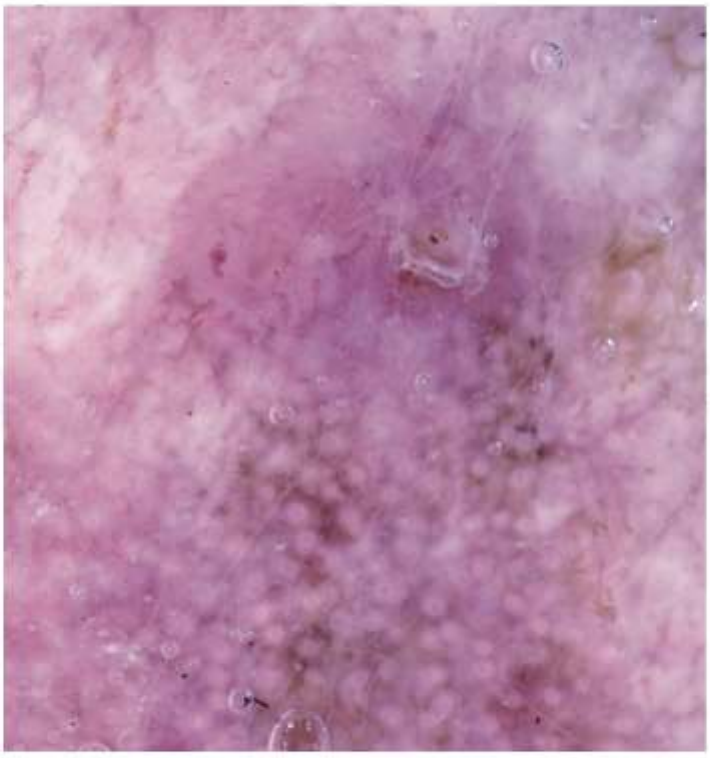

Figure 5.
Histopathologically confirmed basosquamous carcinoma on the border of an AK. Dermatoscopically, two coalescent nodules, both with central ulceration and crust and peripheral dotted and hairpin vessels with white surrounding halo can be seen.

3.3 Dermatoscopic features of AK progressing to SCC

Progression from AK to SCC might follow two pathways. The classical multistep pathway requires proliferation of atypical keratinocytes upwards through the entire epidermis and accumulation of further mutational and cellular events that lead to invasive growth [40]. Nevertheless, the differentiated pathway assumes that invasive SCC may directly arise from a proliferation of atypical basaloid cells of the epidermal basal layer without full-thickness atypia [41].

Dermatoscopic features suggesting progression of AK towards SCC are dotted/glomerular vessels, hairpin vessels, white halos surrounding vessels, ulceration/bleeding, white structureless areas, and white circles surrounding follicles [24]. Appearance of these additional dermatoscopic features is an important clue to perform a diagnostic biopsy even in long-standing AKs, as a great majority of SCCs are associated with preexisting AKs [3] (**Figure 5**).

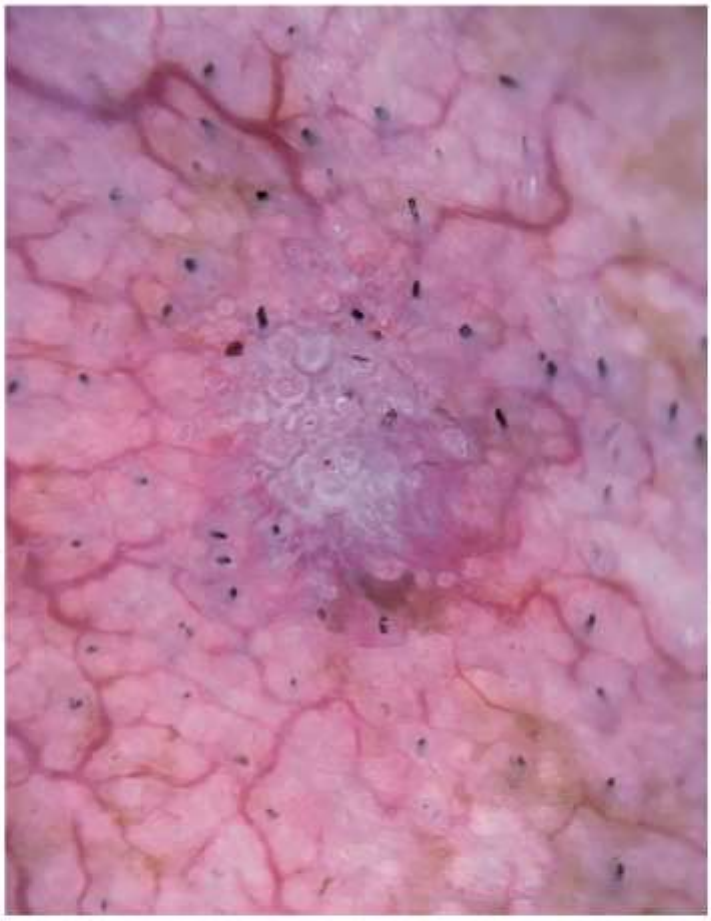

Figure 6.
A lesion on the lower part of the left cheek that clinically presented as an erythematous indurated papule 5 mm in diameter. Dermatoscopically white circles (throughout the lesion), white structureless area (lower part), rosettes (in periphery), and dotted vessels (on the lower part) can be seen. Histopathologically, the basal growth pattern showed filiform papillary elongation protruding into the upper dermal structures in length that exceeds the overlying epidermis.

3.3.1 Characteristics of specific features

3.3.1.1 White circles

On the basis of dermatoscopic–histopathologic correlation, white circles correspond to acanthosis and hypergranulosis of the infundibular epidermis or hyperkeratosis of the infundibular epidermis associated with central keratin plugs [28, 42].

White circles (**Figure 6**) are a specific feature of SCCs and keratoacanthoma-like SCC (KA) and have been shown to be equally common in both and more frequently than in other raised nonpigmented lesions. Moreover, when SCC and KA-like SCC were contrasted with AK and Bowen's disease, the positive predictive value of white circles was 92% in favor of SCC and KA-like SCC [42]. Nevertheless, another study did not find a statistically significant difference between the prevalence of white circles in KA-like SCC and SCC, vs., AK and BD [28]. Other lesions with white circles described are basal cell carcinomas, Bowen's disease, seborrheic keratosis, lichen planus–like keratosis, lichen simplex chronicus, folliculitis, ulcer, chondrodermatitis nodularis helicis, and a dermal nevus [42].

4. Conclusion

Dermatoscopy is a useful tool for the differentiation of AK, IEC, and other non-pigmented facial lesions. The diagnosis is based on the combination of lesion specific factors such as background and follicular structures, vascular patterns, and surface characteristics in addition to information received from the surrounding skin.

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Trešā publikācija

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Article

Expression of p53, p63, p16, Ki67, Cyclin D, Bcl-2, and CD31 Markers in Actinic Keratosis, In Situ Squamous Cell Carcinoma and Normal Sun-Exposed Skin of Elderly Patients

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Abstract: Background: Age and cumulative exposure to ultraviolet (UV) light are primary contributors to skin cancer development. Regulatory proteins within the cell cycle are essential for the homeostasis of squamous epithelium. Methods: This study assessed the expression of immunohistochemical markers p53, p63, p16, Ki67, Cyclin D, Bcl-2, and CD31 in keratinocyte intraepithelial neoplasia (actinic keratosis and squamous cell carcinoma in situ) compared to normal skin. The objective was to distinguish disease-specific changes from those attributable to ageing and sun exposure in elderly skin. Results. Analysis included 22 actinic keratoses (AK), 7 in situ squamous cell carcinomas (SCC), and 8 normal skin biopsies. The mean age was 78.1 years for the AK/SCC group and 73.8 years for controls, with no significant age difference noted between the groups. The AK/SCC group exhibited a higher occurrence of amorphous masses, higher intensity of p53, lower Bcl-2 expression in the epidermis, higher Bcl-2 expression in the dermis, and higher CD31 expression in the dermis, all of which were statistically significant ($p < 0.05$). Conclusions: The study identifies distinct differences in the presence of amorphous masses and the expression levels of p53, Bcl-2, and CD31 between sun-exposed skin and in situ cutaneous squamous cell carcinomas, including actinic keratoses.

Keywords: age; UV damage; immunohistochemistry; cell cycle regulatory markers; skin cancer

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1. Introduction

Cumulative ultraviolet (UV) damage constitutes the principal environmental factor leading to keratinocyte intraepithelial neoplasia and the subsequent emergence of skin cancer. Actinic keratoses (AK), often classed as either precancerous lesions or squamous cell carcinomas (SCCs) in situ, are prevalent conditions in general and dermatological practice [1–5]. These lesions typically develop in older individuals with fair skin. Their prevalence demonstrably increases from the third decade of life and exceeds 90% in individuals beyond 80 years of age [5,6]. Morphologically, AK and SCC are widely regarded as existing on a continuous nosological spectrum but at distinct evolutionary stages [7]. Histopathologically, AK is distinguished by basal keratinocyte atypia, characterized by loss of polarization, cellular crowding, and overlapping. In more advanced stages, this atypia can encompass nearly the entire thickness of the epidermis. AK is stratified into three grades based on the extent of vertical involvement by atypical keratinocytes: Grade I

involves the lower third, Grade II extends to the lower two-thirds, and Grade III affects more than two-thirds of the epidermis without reaching full-thickness [8]. The bowenoid subtype of AK, notable for its full-thickness atypia, complicates differentiation from SCC in situ. Dermatoscopically, bowenoid AK is marked by a uniform distribution of glomerular vessels and the lesion is found within a cancerization field. This contrasts with Bowen's disease, a type of SCC in situ which displays a dermatoscopic pattern of irregularly distributed, clustered vessels, and is typically not associated with field cancerization [5]. Although bowenoid AK is typically described as exhibiting full-thickness dysplasia without adnexal involvement [9], Fernández-Figueras et al. [10] have observed that AK, even when confined to the lower third of the epidermis, may extend into adnexal structures. In this study, lesions with full-thickness involvement are unequivocally classified as SCC in situ.

The p53, p63, p16, Ki67, Cyclin D, and Bcl-2 proteins are essential regulators of the cell cycle [1–5,7], contributing not only to the development and homeostasis of squamous epithelium [11,12] but also to tumor genesis [13,14]. Their expression is modified by both age [15,16] and UV radiation exposure [12,17–19]. Specifically, p53 is a tumor-suppressor protein that interrupts the cell cycle to permit DNA repair [20]. The mutation and subsequent inactivation of p53 mark a critical early pathogenic step in SCC development, as this dysfunction allows cells to evade apoptosis, leading to the clonal proliferation of the mutated cells [21]. Multiple studies have documented the overexpression of p53 in precancerous skin lesions and SCC, as well as in skin after sun exposure, with elevated p53 levels recognized as a biomarker of recent sun exposure [22]. Increased p53 has been associated with progression from AK to SCC [13].

p63, a member of the p53 family of transcription factors, plays a pivotal role in keratinocyte differentiation across various stages. While p63 is abundantly expressed in the basal and suprabasal layers of normal epidermis, its expression diminishes in the upper spinous layer and is absent in the granular and cornified layers. Aberrant p63 expression is a hallmark of SCC across different organ sites, contributing to oncogenesis by disrupting numerous cell processes, including the inhibition of oncogene-induced keratinocyte senescence and reducing the function of other p53 family members [23,24].

Cyclin D1 plays an important role for the G1-S phase transition within the cell cycle. It activates CDK4 and CDK6, leading to the phosphorylation of the retinoblastoma protein, which in turn initiates transcription and activates proteins involved in the G1 checkpoint passage and S phase entry. Cyclin D1 overexpression truncates the G1 phase resulting in abnormal proliferation [25]. In situ SCCs have demonstrated more prevalent diffuse immunostaining of cyclin D1 compared to AK, suggesting a role in AK progression [26].

The p16 protein, encoded by the CDKN2A gene, impedes the cell cycle's progression from the G1 to S phase by binding to and inactivating CDK4 and CDK6. It interacts with retinoblastoma and p53 tumor-suppressor genes in its role as a cell cycle inhibitor [27]. UV radiation activates p16, and p16-expressing cells have been detected in AK lesions. In a mouse model, persistent p16 expression in a subset of epidermal cells was shown to induce hyperplasia and dysplasia, promoting tumor formation after mutagenesis [16].

Ki67, a nuclear antigen, is a marker of cell proliferation across various cycle phases (G1, S, G2, M) and is absent in the quiescent phase (G0). Ki67 expression correlates with mitotic count and is employed as a surrogate marker for assessing the rate of proliferation, which is crucial for the diagnosis, classification, and prognosis of various neoplasms. SCC arising from AK through the differentiated pathway exhibits significantly higher Ki67 scores compared to the classical pathway. This increased proliferation also explains the progression of AK from a lesion with basal atypia into a bowenoid neoplasia with complete replacement of the epidermis by atypical cells and frequent mitoses in all epithelial layers [28].

The BCL-2 gene (B-cell lymphoma-2) encodes a protein that inhibits apoptosis. Within normal skin, Bcl-2-positive basal cells serve as a reservoir for the renewal of squamous epithelium [29]. In sun-exposed skin, Bcl-2 prevents UV-induced cell death [30]. An upregulation of this anti-apoptotic Bcl-2 protein is implicated in the progression from

AK to SCC [12]. Additionally, Bcl-2 can label inflammatory cell infiltrate [31] and, within the complex process of cancer development, the extent of dermal infiltrate correlates significantly with keratinocyte atypia, mitotic count, and adnexal involvement [32].

CD31 is recognized as a highly sensitive and specific endothelial marker in paraffin tissue samples [33]. Neovascularisation plays a critical role in tumor development and progression. Studies have previously demonstrated a significant increase in the microvascular area corresponding with the transition from AK to cutaneous SCC [34].

In this cross-sectional study, we compared the expression of immunohistochemical markers—p53, p63, p16, Ki67, Cyclin D, Bcl-2, and CD31—between a group presenting with intraepithelial keratinocyte neoplasia, specifically AK and SCC in situ (experimental group), and a control group with normal-appearing skin. Our objective was to discern which marker associations are unique to the disease and which can be anticipated in elderly skin exposed to sunlight. To our knowledge, this study is the first to evaluate these seven immunohistochemical markers concurrently in AK and SCC in situ, and healthy sun-exposed skin. The findings derived from this study are anticipated to lay the groundwork for subsequent investigations into the relationship between immunohistochemical marker expression and clinical as well as dermatoscopic characteristics.

2. Materials and Methods

2.1. Patient Population

Considering the increased prevalence of AK and SCC among the aging population, our study included elderly fair-skinned individuals. These patients underwent examination by a board-certified dermatologist who diagnosed and selected the biopsy sites for AK or SCC in situ. The extent of sun damage was quantified using the AK field assessment scale [35] according to which all participants exhibited moderate to severe photodamage, necessitating more frequent follow-up consultations. Evaluated parameters included the count of AK lesions, evidence of field cancerization, wrinkles, pigmentation changes, telangiectasia, and cutaneous atrophy. Our patient sample did not have significant occupational sun exposure. However, given the prevalence of outdoor activities in the Latvian population and the low use of sunscreen among the elderly cohort, incidental sun exposure was considered noteworthy.

2.2. Tissue Samples

A 3 to 4 mm punch biopsy was taken from lesions clinically and dermatoscopically diagnosed as AK or SCC in situ. These samples underwent routine histopathological evaluation and were included in the study following confirmation of the diagnosis by a study-unrelated pathologist. Control biopsies were prospectively collected from the maxillofacial surgery department specifically from lateral margins of elliptical excisions or remnant tissues from flap preparations. In total, the following number of biopsies were included: 22 from AK, 7 from SCC in situ, and 8 control biopsies. All patients from whom samples were obtained were identified as Caucasian, and all biopsies originated from facial tissue.

2.3. Histopathological and Immunohistochemical Analyses

The extent of atypical keratinocytes within the epidermis was stratified into three grades, as delineated by Röwert-Huber et al. [4]. Similarly, the basal growth pattern was assessed and categorized into three grades (protruding I–III) following the criteria established by Schmitz et al. [36].

Immunohistochemical staining was carried out on formalin-fixed paraffin-embedded tissue using both a DAKO Autostainer Plus (DAKO, Glostrup, Denmark) and Ventana Benchmark XT automated immunostainer (Ventana Medical System, Tuscon, AZ, USA). Detailed information regarding the monoclonal antibodies, clones, dilutions, incubation times, and manufacturers is presented in Table 1. All staining procedures included the use of appropriate controls.

Table 1. List of antibodies and detection methods used in the study.

Antibody	Clone	Dilution	Incubation Time	Source
p53	Do-7	Ready-to-use	30 min	DAKO
p63	A4A	Ready-to-use	20 min	Ventana
p16	E6H4	Ready-to-use	20 min	Ventana
Ki67	MIB1	Ready-to-use	16 min	DAKO
Cyclin D	EP12	Ready-to-use	30 min	DAKO
Bcl-2	124	Ready-to-use	30 min	DAKO
CD31	JC70A	Ready-to-use	30 min	DAKO

Nuclear staining for p16, p53, and p63 was deemed positive, regardless of any associated cytoplasmic staining. The expression levels of p16 and p53 were semi-quantitatively assessed using predefined thresholds: <1%, 1–30%, 30–50%, and >50% for p16 and weak staining (if any), <5%, 5–50%, and >50% for p53. The epidermal distribution of p53, p63, Ki67, CyclinD, and Bcl-2 staining was categorized similarly to the extent of cellular atypia observed in hematoxylin and eosin-stained sections, with five distinct groups: no expression, expression in the lower third, expression up to the lower two-thirds, expression above two-thirds, and full-thickness expression. CD31 expression was quantified by manually counting positively stained vessels across three 20× magnification fields and semi-quantitatively classified into three categories: isolated, scattered perivascular inflammatory cells; foci of moderately pronounced inflammation; and areas of pronounced inflammation.

2.4. Statistical Analysis

The independent sample T-test was employed to evaluate the age difference between the experimental and control groups, predicated on the data's normal distribution, verified by the Shapiro–Wilk test and normal Q-Q plots, and homogeneity was confirmed by Levene's test. Fisher's exact test was performed to investigate the association between the patterns of expression. The $p < 0.05$ was considered statistically significant. All statistical analyses were performed using Jamovi Version 2.3.28, retrieved from https://www.jamovi.org, accessed on 11 July 2023.

2.5. Ethics

All patients provided their informed written consent before the biopsies were taken. The study conformed to the ethical standards of the Declaration of Helsinki and received approval from the relevant local ethics committees.

3. Results

Table 2 summarizes the demographic, pathological, and immunohistochemical characteristics. Mean age exceeded 73 years with no significant difference between experimental and control groups ($p > 0.05$). Both groups had a female predominance.

Sunscreen use was evaluated within the patient cohort. Among female participants, 27.3% reported consistently using sunscreen on sunny days. Conversely, all male participants denied any sunscreen use. However, this observed difference in sunscreen usage between genders was not statistically significant.

As expected, our findings revealed that there are statistically significant differences ($p < 0.05$) in the presence and extent of cellular dysplasia, as well as the degree of protruding, between AK/SCC samples and control biopsies. These differences are markers for distinguishing AK/SCC lesions.

Table 2. Distribution of histopathological and immunohistochemical signs of biopsied lesions.

	Cases, Total (%)	Controls, Total (%)	*p* Value
Total number of cases included	29 (100%)	8 (100%)	
Mean age (±SD)	78.1 (±5.8) years	73.8 (±6.3) years	$p = 0.076$
Gender:			
Males	7 (24.1%)	3 (37.5%)	$p = 0.655$
Females	22 (75.9%)	5 (62.5%)	
Extent of atypical keratinocytes in the epidermis [4]			
No atypical keratinocytes	0 (0%)	8 (100%)	$p < 0.001$ *
In the lower 1/3	4 (13.8%)	0 (0%)	
Up to lower 2/3	14 (48.3%)	0 (0%)	
Above 2/3	4 (13.8%)	0 (0%)	
Full thickness	7 (24.1%)	0 (0%)	
Protruding [36]			
None	5 (17.2%)	8 (100%)	$p < 0.001$ *
Crowding (pro I)	7 (24.1%)	0 (0%)	
Budding (pro II)	3 (10.3%)	0 (0%)	
Papillary sprouting (pro III)	14 (48.3%)	0 (0%)	
Presence of amorphic masses	21 (72.4%)	0 (0%)	$p < 0.001$ *
p16 semiquantitative expression			
<1%	13 (44.8%)	4 (50%)	$p = 0.865$
1–30%	10 (34.5%)	4 (50%)	
30–50%	3 (10.3%)	0 (0%)	
>50%	3 (10.3%)	0 (0%)	
p16 distribution			
Negative	13 (44.8%)	4 (50%)	$p = 1.0$
Patchy	13 (44.8%)	4 (50%)	
Patchy/Diffuse	3 (10.3%)	0 (0%)	
p53 semiquantitative expression			
Weak staining (if any)	5 (17.2%)	1 (12.5%)	$p = 0.740$
<5%	2 (6.9%)	0 (0%)	
5–50%	11 (37.9%)	5 (62.5%)	
>50%	11 (37.9%)	2 (25.0%)	
p53 distribution in the epidermis			
No expression	6 (20.7%)	1 (12.5%)	$p = 0.885$
In the lower 1/3	9 (31.0%)	3 (37.5%)	
Up to lower 2/3	4 (13.8%)	2 (25.0%)	
Above 2/3	9 (31.0%)	2 (25.0%)	
Full thickness	1 (3.4%)	0 (0%)	
p53 staining intensity			
No staining	6 (20.7%)	0 (0%)	$p = 0.036$ *
Weak staining	10 (34.5%)	7 (87.5%)	
Strong staining	13 (44.8%)	1 (12.5%)	

Table 2. *Cont.*

	Cases, Total (%)	Controls, Total (%)	*p* Value
p63 distribution in the epidermis			
Up to lower 2/3	5 (17.2%)	1 (12.5%)	$p = 1.0$
Above 2/3	23 (79.3%)	7 (87.5%)	
Full thickness	1 (3.4%)	0 (0%)	
Cyclin D distribution in the epidermis			
In the lower 1/3	11 (37.9%)	0 (0%)	$p = 0.075$
Up to lower 2/3	14 (48.3%)	7 (87.5%)	
Above 2/3	4 (13.8%)	1 (12.5%)	
Ki67 distribution in the epidermis			
In the lower 1/3	14 (48.3%)	7 (87.5%)	$p = 0.398$
Up to lower 2/3	11 (37.9%)	1 (12.5%)	
Above 2/3	2 (6.9%)	0 (0%)	
Full thickness	2 (6.9%)	0 (0%)	
Bcl-2 distribution in the epidermis			
No expression	16 (55.2%)	0 (0%)	$p = 0.014$ *
In the lower 1/3	8 (27.6%)	5 (62.5%)	
Up to lower 2/3	4 (13.8%)	2 (25.0%)	
Above 2/3	1 (3.4%)	1 (12.5%)	
Bcl-2 semiquantitative expression in the epidermis			
No or almost no cells	16 (55.2%)	0 (0%)	$p = 0.015$ *
Weak (<10%)	6 (20.7%)	4 (50.0%)	
Moderately positive (10–25%)	3 (10.3%)	2 (25.0%)	
Highly positive (>25%)	4 (13.8%)	2 (25.0%)	
Bcl-2 subepidermal infiltration			
Weak or none	2 (6.9%)	7 (87.5%)	$p < 0.001$ *
Foci with more pronounced	22 (75.9%)	1 (12.5%)	
Foci with very pronounced	5 (17.2%)	0 (0%)	
CD31 expression in dermal capillaries			
Up to 20 positive capillaries at 20× magnification	1 (3.4%)	2 (25.0%)	$p = 0.174$
20–40 positive capillaries at 20× magnification	19 (65.5%)	5 (62.5%)	
Above 40 positive capillaries at 20× magnification	9 (31.0%)	1 (12.5%)	
CD31 semiquantitative dermal expression			
Separate scattered positive vessels	2 (6.9%)	3 (37.5%)	$p = 0.011$ *
Foci with moderately pronounced expression	8 (27.6%)	5 (62.5%)	
Foci with pronounced expression	12 (54.5%)	0 (0%)	

* p value < 0.05 was considered statistically significant.

Furthermore, statistically significant differences ($p < 0.05$) were observed between the experimental and control groups in various evaluations, including the presence of amorphous masses, the intensity of p53, the extent of Bcl-2 in epidermal layers, quantitative expression of Bcl-2 in the epidermis, subepidermal infiltration of Bcl-2, and the amount of CD31 dermal infiltrate. Representative images of the expression of immunohistochemical markers can be found in Figures 1 and 2.

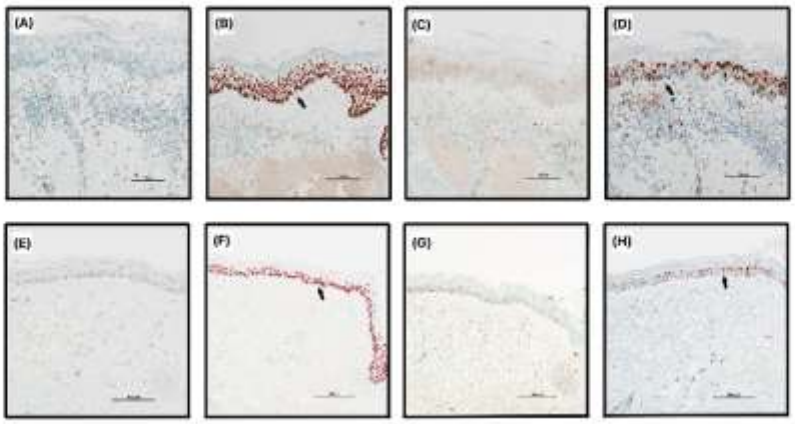

Figure 1. Immunohistochemical expression of p53, p63, p16, and Cyclin D in actinic keratosis (**A**–**D**) and control (**E**–**H**) skin. Black arrows indicate strong positive cell staining. (**A**) expression of p53 in actinic keratosis (AK) with weak staining if any; (**B**) expression of p63 in AK, distribution above 2/3 of epidermal thickness; (**C**) expression of p16 in AK with 1–30% nuclear staining; (**D**) expression of Cyclin D in AK, distribution up to 2/3 of epidermal thickness; (**E**) expression of p53 in control skin (CS) with <5% nuclear staining; (**F**) expression of p63 in CS, distribution above 2/3 of epidermal thickness; (**G**) expression of p16 in CS with <1% nuclear staining; (**H**) expression of Cyclin D in CS, distribution up to 2/3 of epidermal thickness.

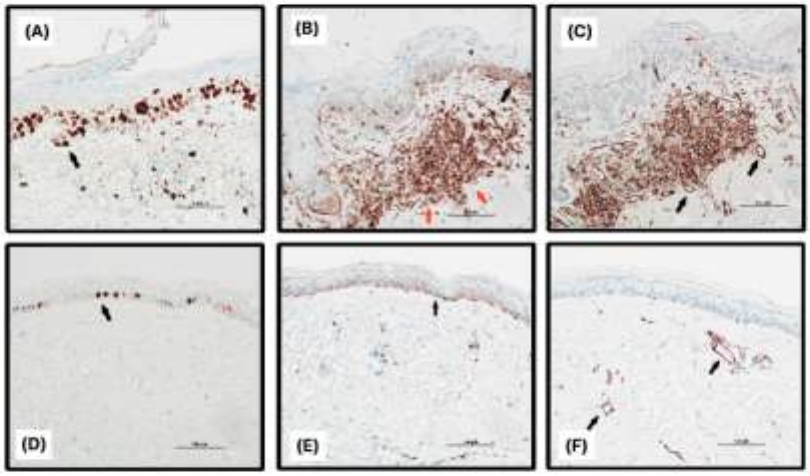

Figure 2. Immunohistochemical expression of Ki67, Bcl-2, and CD31 in actinic keratosis (**A**–**C**) and control (**D**–**F**) skin; (**A**) expression of Ki67 in actinic keratosis (AK), distribution up to 2/3 of epidermal thickness; (**B**) expression of Bcl-2 in AK, distribution up to 2/3 of epidermal thickness, foci with pronounced subepidermal infiltration (red arrows); (**C**) expression of CD31 in AK, visible pronounced expression; (**D**) expression of Ki67 in control skin (CS), distribution of up to 1/3 of epidermal thickness; (**E**) expression of Bcl-2 in CS, distribution up to 2/3 of epidermal thickness, weak or none subepidermal infiltration; (**F**) expression of CD31 in CS, few visible positive capillaries. Black arrows indicate positive cell staining in the epidermis (**A**,**B**,**D**,**E**) and positive endothelial staining (**C**,**F**).

4. Discussion

Exposure to ultraviolet (UV) light is the primary environmental factor responsible for the onset and progression of skin cancer. Consequently, areas of the skin that are frequently exposed to sunlight, such as the face, are the most common sites for the development of skin cancer [5,37,38].

In this study, we conducted a comparative analysis of histopathological and immunohistochemical markers between samples of normal skin and intraepidermal keratinocyte neoplasia, specifically AK and in situ SCC, which were collected prospectively. Additionally, we evaluated the potential impact of age and UV exposure on the expression of immunohistochemical markers by focusing on a cohort of elderly patients [15,17,39]. The patient cohort in our study was predominantly female. This predominance is noteworthy given that AKs are typically more frequently diagnosed in men, who, due to higher rates of baldness, often have greater skin exposure to the sun. Nonetheless, our findings align with other studies that have similarly reported a higher incidence of AKs among women [40–42]. Furthermore, the predominance of women in our study sample may be attributed to the differences in life expectancy between genders in Latvia, where in 2021, it was 68.2 years for men and 77.9 years for women. This is particularly relevant given that the mean age of our patient cohort was 78.1 years [43]. Contrary to the common perception that women tend to use a broader range of cosmetic products, including sunscreens, more frequently than men, our study's findings did not show a statistically significant gender difference in sunscreen use. However, the use of moisturizing creams exhibited a notable gender disparity, with 66.7% of women in our cohort using these products compared to none of the men.

In summary, our study identified differences in the prevalence of amorphous masses, the intensity of p53 staining, and the levels of Bcl-2 and CD31 expression.

In the majority of AK and SCC cases, we identified amorphous masses, a finding not seen in any of the control biopsies. This observation aligns with findings from previous research, which reported a high incidence of severe solar elastosis in cutaneous SCC [44,45]. Severe solar elastosis, marked by the substitution of dermal collagen with elastotic material, results from prolonged and excessive UV damage [44].

The abnormal expression of p53 is considered a marker of premalignant lesions and plays a central role in the development of SCC [20]. A notable increase in p53 staining correlates with aging and exposure to sunlight, while its expression diminishes with the consistent application of sunscreen [37]. Essentially, progression from AK to SCC is associated with increased p53 staining [13,15,46]. Simultaneously, research by Piipponen et al. identified a fraction of AK, in situ SCC (8%), and cutaneous SCC (11%) lesions that were p53 negative, suggesting a possible nonsense mutation in the tumor protein 53 gene leading to the absence of functional p53 protein [47]. Similarly, Neto et al. found a higher incidence of SCC adjacent to AK lesions where less than 25% of cells were p53 positive [20]. In our study, we saw a slightly higher intensity of p53 staining in the AK/SCC cohort, aligning with the literature, while no notable differences were found in the pattern or extent of p53 expression across the epidermal layers between the two groups. Similarly, as observed by Neto et al. [20] and Piipponen et al. [47], not all AK cases in our study showed p53 expression. More than 5% immunoreactivity to p53 was observed in 75.8% of the AK/SCC samples and the staining was considered strong in 44.8% of the cases.

Bcl-2 is an antiapoptotic molecule located in the mitochondrial membrane. Alteration of its function can promote cancer development. Prior research has noted an increase in Bcl-2 expression in AK [48]. Additionally, studies have indicated a significantly higher prevalence of Bcl-2-positive tumor cells in SCC compared to asymptomatic AK. Furthermore, a stepwise increase in Bcl-2 expression has been documented from asymptomatic AK to inflamed AK and subsequently to SCC, suggesting a pathway of progression through inflamed AK [46]. Our results agree with previous studies, showing higher expression of Bcl-2 in the epidermis of both AK and in situ SCC samples. Moreover, Bcl-2 stained dermal inflammatory cells and inflammation is associated with a progression of AK to SCC due to its role in generating reactive oxygen species and fostering immune responses, cellular

transformation, survival, proliferation, invasion, angiogenesis, and metastasis [48–50]. Our results also revealed that both the Bcl-2 subepidermal infiltrate and CD31 expression, which is the most sensitive and specific endothelial marker in paraffin-embedded sections [33], are elevated in the AK/SCC samples compared to controls.

One of the strengths of this study is that all the biopsies were consistently taken from the facial area. The results obtained allow us to further analyze associations between dermatoscopical features and p53, CD31, and Bcl-2 expression within the same cohort and avoid analyzing markers that showed no difference with the control group. Such insights have the potential to expand our understanding of the interplay between dermatoscopic characteristics and immunohistochemical marker expression. This could also potentially enhance the diagnostic precision of dermoscopy by pinpointing features that correlate with more aggressive histological and immunohistochemical profiles, such as adnexal involvement, protruding, and increased expression of p53, CD31, and Bcl-2.

5. Conclusions

The presence of amorphous masses and the expression of p53, Bcl-2, and CD31 differ between sun-exposed skin and cutaneous squamous cell carcinomas in situ, including actinic keratoses.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Riga Stradiņš University (protocol code 50/22.02.2018. date of approval 22 February 2018 and protocol code 22-2/548/2021, date of approval 3 December 2021) and Riga East University Hospital (protocol code ZD/08-07/01-21/18, date of approval 7 December 2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data is available upon reasonable request. The data are not publicly available due to the continuous nature of the study.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Dermatoscopic and histopathologic correlations of facial keratinocyte neoplasia.

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Introduction and objectives. Diagnostics of all skin conditions relies on clinical, instrumental, and histopathological features. Dermatoscopy has become a popular and easy to use hand-held device that reveals significant structures unseen with a naked eye. Nevertheless, microscopical analysis, including histopathology, is commonly considered as a gold standard in determining the presence and nature of a disease. In adjunction, immunohistochemistry (IHC) is used in histology to detect the presence of specific protein markers that can assist with accurate tumour classification and diagnosis. Altogether the studies on correlations of dermatoscopical and histopathological features are lacking.

Materials and methods. In a prospective sample of 29 lesional and 8 control biopsies from facial skin of elderly patients dermatoscopical, histopathological and immunohistochemical features with the following markers were compared: p53, p63, p16, Ki67, Cyclin D, Bcl2 and CD31. The lesional skin was taken as 3-4 mm punch biopsy from actinic keratoses (AK) or squamous cell carcinomas in situ.

Results. Mean age was slightly higher for case patients, but the difference was not significant (78.1 vs. 73.8 years, p=0.076, independent sample T test). Statistically significant differences between cases and controls were found in the following assessments: presence of amorphous masses, staining intensity of p53, Bcl-2 expression, and extent of CD31 dermal infiltrate. Amorphous masses were present in all cases of dermatoscopically typical AK, while only in part of lesions with dermatoscopically suspicious signs (p=0.009, Ficher's Exact Test).

Other features, like vascular signs of dermatoscopically typical AK were also associated with presence of amorphous masses, in particular absence of suspicious vessels (p=0.014, Ficher's Exact Test) and presence of small arborising vessels (p=0.009, Ficher's Exact Test). Intensity of p53 was not associated with any of dermatoscopical signs studied. Increased Bcl2 subepidermal expression was associated with absence of follicles (p=0.007, Ficher's Exact Test). Dermatoscopically typical AK had lower expression of CD31 (p=0.043, Ficher's Exact Test).

Conclusions. Development of actinic keratosis is strongly associated with sun damage, while at certain moment other mechanisms are initiated and promote cancer development. Immunohistochemically seen inflammation and neovascularisation markers are associated with some dermatoscopical signs.

Piektā publikācija

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DERMOSCOPIC MONITORING OF SHINY WHITE STREAKS DURING TOPICAL TREATMENT OF ACTINIC KERATOSIS

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Abstract

Dermoscopic monitoring of shiny white streaks during topical treatment of actinic keratosis

Key Words: *shiny white streaks, chrysalis structures, actinic keratosis, dermoscopy*

Background and Objectives. It has been shown that dermoscopy improves diagnostic accuracy of a clinician. Nevertheless, data on dynamics of dermoscopically seen structures and treatment impact on them is scarce; therefore, we chose to monitor shiny white streaks (SWS), during topical treatment of actinic keratoses (AK).

Materials and Methods. AK lesions located on face or scalp were treated with either topical 5% 5-fluorouracil cream (5FU) or daylight photodynamic therapy with methyl aminolevulinate (DL-PDT). Dermoscopic assessments were performed before start of therapy, at peak inflammatory phase and one-month post-treatment (Follow-up visit).

Results. Of 38 lesions followed, before start of therapy SWS were present in 18 (47%) lesions. In five cases (13%) SWS remained present through all visits. In three cases (8%) SWS were present until the Follow-up visit. In ten (26%) AKs SWS disappeared at the peak inflammatory phase, but in four (11%) of those SWS later reappeared. Of 20 (53%) lesions without SWS at the first visit, only one (3%) developed SWS at a Follow-up visit.

Conclusions. SWS seem to be variable structures that can be unseen in lesions with therapy induced inflammation, disappear following topical treatment of AK and sometimes appear for the first time post-treatment. It could be important to take into consideration the dynamics of SWS when assessing their presence.

Kopsavilkums

Balto spīdīgo strēļu dermatoskopiskā monitorēšana aktīnisko keratožu lokālās terapijas laikā

Atslēgvārdi: *Baltās spīdīgās strēles, Krizāla struktūras, aktīniskās keratozes, dermatoskopija*

Ievads un mērķis. Pētījumi liecina, ka klīniskajā praksē dermatoskopija uzlabo ārsta spēju precīzi diagnosticēt ādas veidojumus. Dermatoskopiskās diagnozes pamatā ir specifisku struktūru atpazīšana, tomēr atsevišķas dermatoskopiskās struktūras laika gaitā mainās un trūkst datu par to dinamiku, tai skaitā, lokālas terapijas ietekmi uz dermatoskopiskajām struktūrām. Šī pētījuma mērķis bija dinamikā novērtēt baltās spīdīgās strēles (BSS) lokālas aktīnisko keratožu terapijas laikā.

Materiāli un metodes. Aktīniskās keratozes sejas un skalpa ādā tika ārstētas vai nu ar 5% 5-fluoruracila krēmu, vai ar dienas gaismas fotodinamisko terapiju izmantojot metilaminolevulināta krēmu. Veidojumi tika dermatoskopiski novērtēti pirms terapijas (1. vizīte), izteikta terapijas radīta ādas iekaisuma laikā (2. vizīte) un vienu mēnesi pēc terapijas (pēcterapijas vizīte).

Rezultāti. No 38 aktīniskajām keratozēm, kas atbilda iekļaušanas kritērijiem un tika novērtētas dinamikā, BSS tika konstatētas 18 (47%) veidojumiem. No tiem piecos gadījumos (13%) BSS saglabājās visās vizītēs. Trijos gadījumos (8%) BSS saglabājās līdz pēcterapijas vizītei, kad tās vairs nebija saskatāmas. Desmit (26%) gadījumos BSS izzuda 2. vizītē, kas atbilda visizteiktākajam terapijas radītajam ādas iekaisumam, bet četros gadījumos (11%) BSS vēlāk atjaunojās pēcterapijas vizītē. No 20 (53%) veidojumiem bez BSS 1. vizītē tikai vienam veidojumam (3%) attīstījās BSS, kas tika konstatēts pēcterapijas vizītē.

Secinājumi. BSS ir šķietami variablas struktūras, kas lokālas aktīnisko keratožu terapijas laikā var izzust terapijas radītā iekaisuma fāzē un pēc terapijas, kā arī dažkārt parādīties pēc terapijas. BSS dinamika būtu jāņem vērā analizējot to sastopamību.

Introduction

Shiny white streaks (SWS) also known as chrysalis or crystalline structures are white, perpendicular, few millimeters long lines that are only visible in a polarized light dermoscopy (Kittler et al. 2016). These structures, considered as a dermoscopic sign of dermal fibrosis, are caused by polarization of thickened hyaline fibrous bundles (Pizzichetta et al. 2014; Haspeslagh et al. 2016), and have been reported in a variety of skin lesions, including actinic keratosis (AK) (Balagula et al. 2012; Liebman et al. 2012). At present, the clinical significance of SWS has been associated with melanocytic skin lesions, namely a sign of 10-fold increased risk of malignancy (Shitara et al. 2014). The clinical significance of SWS in non-pigmented lesions is yet to be defined. Additionally, data on permanence of SWS and treatment impact on SWS is lacking. Therefore, we used topical treatment of AK as model to dermoscopically monitor dynamics of SWS.

Materials and methods

Presence of SWS was continuously assessed in eight patients with AK lesions located on face or scalp and treated with either topical 5% 5-fluorouracil cream (5FU) or daylight photodynamic therapy with methyl aminolevulinate (DL-PDT). Diagnosis of AK was made according to clinical and dermoscopic signs and confirmed histologically in each patient in a single 4mm punch biopsy specimen. Biopsied lesions were not included into assessment to exclude possible change in SWS due to scar formation. In addition, only lesions that lacked superficial scale and did not develop scales or erosions during therapy were included for continuous assessment of SWS. Dermatoscopic assessments of lesions treated with 5FU were performed before start of therapy (Visit 1), at peak inflammatory phase corresponding to three or four weeks of 5FU usage (Visit 2) and one-month post-treatment (Follow-up visit). Dermatoscopic assessments of lesions treated with DL-PDT were performed before start of therapy (Visit 1), at peak inflammatory phase corresponding to one-day post-treatment (Visit 2) and one-month post-treatment (Follow-up visit). All dermatoscopic pictures were taken in a polarized light mode with FotoFinder Systems GmbH medicam 1000 device. All dermatoscopic evaluations were performed simultaneously by two physicians specializing in dermoscopy (A.B. and E.O.).

Results

Eight patients had a total number of 90 AKs that were dermoscopically monitored. For continuous assessment of SWS only 38 lesions were suitable; the rest were excluded due to reasons stated above, mainly presence of superficial scale or development of erosions during therapy. Presence of SWS by visit number is depicted in Fig. 1. Before start of therapy SWS were present in 18 (47%) of AKs. Of those, in 5 cases (13%) SWS remained present in all visits; in 3 cases (8%) SWS were present until Follow-up visit, when SWS were no more visualized. In 10 (26%) AKs SWS disappeared at the Visit 2, corresponding to treatment induced inflammation, but in 4 (11%) of

those, SWS reappeared at a Follow-up visit (Fig.2). Of 20 (53%) lesions without SWS at the first visit, only 1 (3%) developed SWS at a Follow-up visit.

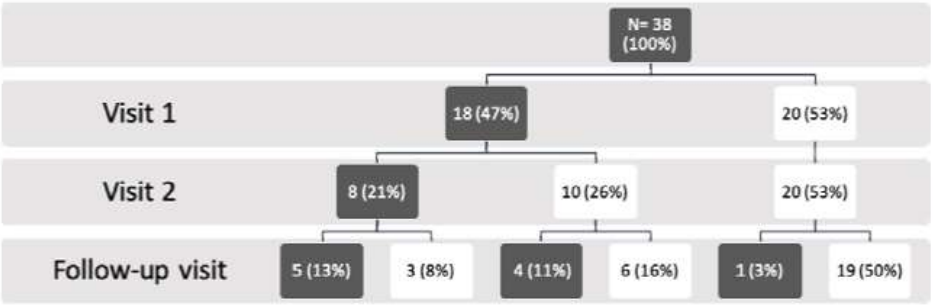

Figure 1. **Presence of SWS by visit number. Dark grey cells indicate presence of SWS, white cells – absence of SWS. Visit 1 – before start of therapy; Visit 2 – at treatment induced inflammatory phase; Follow-up visit – one-month post-treatment**

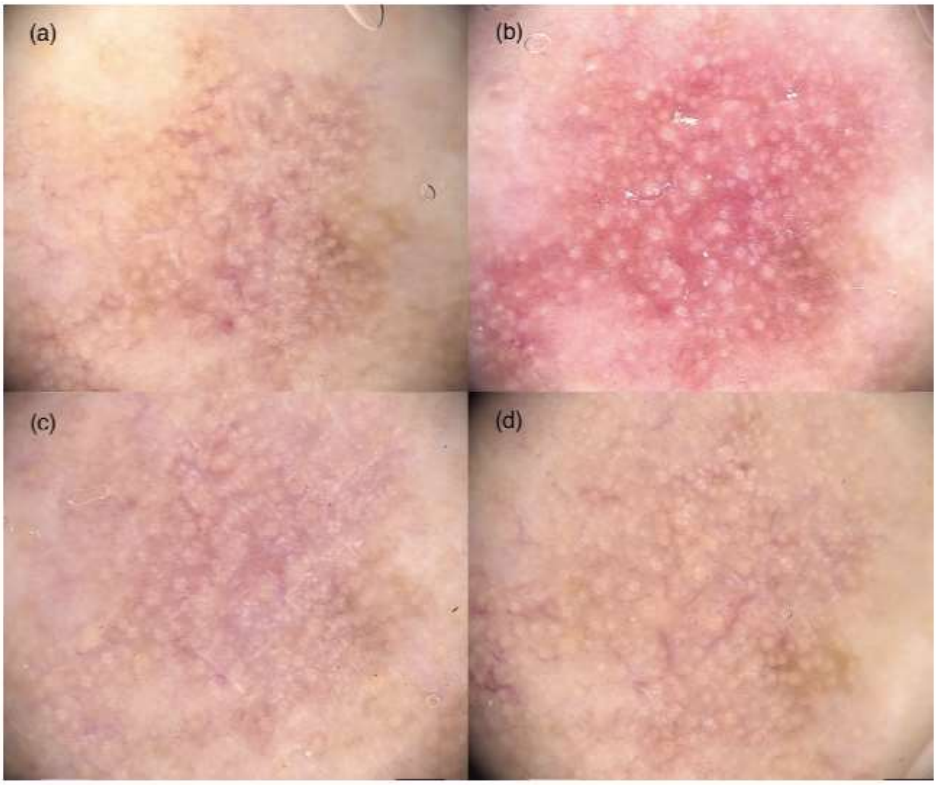

Figure 2. **Dermatoscopic monitoring of SWS in AK treated with DL-PDT. SWS are visualized at Visit 1 (a), 1 week after therapy (c) and to a smaller extent at Follow-up visit (d); SWS are lacking at Visit 2 (b), when therapy induced inflammation is observed**

Discussion

Dermatoscope is an easy to use handheld device, that renders magnification and removes skin surface reflection, thus visualizing morphologic structures unseen with a naked eye (Pan et al. 2008). It has been shown to improve diagnostic accuracy of both pigmented and non-pigmented skin lesions and at times to provide relevant prognostic information through known dermoscopic-histopathologic correlations (Sinz et al. 2017). Common dermoscopic signs of AK are white-to-yellow surface scale, red pseudonetwork, which is formed by perifollicular erythema often combined with linear-wavy telangiectasia, targetoid-like hair follicles which are formed by yellowish keratotic plugs that fill and white halo that surrounds hair follicles, and rosette sign (Lee et al. 2014; Zalaudek et al. 2006). Although the clinical significance of SWS in AK is not yet fully established, SWS, as seen in our case series, is a common dermoscopic feature of AK with a higher prevalence than previously reported by *Balagula et al.* and *Liebman et al.* (Balagula et al. 2012; Liebman et al. 2012). This observation could be explained by selection of AK lesions, as lesions with scales that could possibly hide SWS were excluded from our study. Noteworthy, scale is a common feature of AK, with a prevalence of 79.4 – 85% (Lee et al. 2014; Zalaudek et al. 2006). In addition, scales, crusts and erosions commonly develop with topical treatment due to destruction of atypical keratinocytes and such lesions were also excluded. Another reason for high prevalence of SWS in our study was that even a small amount of SWS were counted as a positive feature and FotoFinder Systems medicam 1000 device offers higher magnification and resolution in comparison with handheld devices. In our study, despite the small sample size, it was possible to determine several possible scenarios of treatment impact on SWS. First, although successful therapy is usually associated with disappearance of dermoscopic signs, SWS can remain present through all treatment stages or even appear at a 1-month post-treatment visit without other dermoscopic signs of AK. As SWS is not a required feature for AK, permanence of SWS is not a counter-condition to treatment success. Second, as other dermoscopic structures, SWS can disappear during or after therapy and finally, SWS can temporarily disappear during treatment induced inflammation and reappear thereafter. This last observation was present in 13% of lesions analyzed, and although the exact reason for such phenomenon is not clear yet, it leads us to speculate that SWS might also be hidden in other clinically clearly erythematous lesions, not limited to AK.

To the best of our knowledge, this is the first study in which SWS are continuously monitored. In conclusion, we would like to emphasize that SWS seem to be variable structures that can be unseen in lesions with therapy induced inflammation, disappear following topical treatment of AK and sometimes appear for the first time after treatment. It could be important to take into consideration the dynamics of SWS when assessing their presence.

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Case Report

Treatment of Actinic Keratoses Facilitates Dermatoscopic Diagnosis of Early Basal Cell Carcinoma: A Case Report and Review

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Keywords
Actinic keratosis · Dermoscopy · Basal cell carcinoma · Case report

Abstract
Chronic exposure to ultraviolet radiation induces gradual changes in cutaneous morphology, which with increasing damage leads to the appearance of cancerous skin lesions. Among them, basal cell carcinomas (BCCs) and actinic keratoses (AKs) are the most common entities. Both lesions often develop as two separate lesions in a single individual at a conspicuous distance, close proximity or as collision lesions, which are characterized by the coexistence of both cancers in the same anatomical site. Collision lesions in which AK precisely overlies BCC is a rarely reported entity. We report a case where the presence of BCC was dermatoscopically detected after an overlying AK was treated with topical chemotherapy, thus indicating that treatment of AK allows better visualization of other underlying malignancies.

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Introduction

Actinic keratoses (AKs), which can be considered as squamous cell carcinomas (SCCs) in situ, and basal cell carcinomas (BCCs) are the most common ultraviolet radiation-induced keratinocyte skin cancers with high and continuously increasing prevalence. Recent European studies have estimated that AKs affect 25.3% of outpatients above the age of 30 years visiting a dermatologist or a general practitioner [1, 2]. Similarly, the incidence of BCCs is increasing every year with the cumulative risk in Belgium assessed as 11% before the age of 75 years [3, 4]. Both AK and BCC share a substantial amount of environmental and individual risk factors including ultraviolet radiation, fair skin, low ability to tan, higher age, male sex, personal or family history of skin cancer and immunosuppression [1, 3, 5, 6]. Both are markers of cutaneous photodamage and the presence of AK and BCC in a single individual is not uncommon; moreover, the presence of AK has been shown to increase the risk of BCC by approximately 3 times [7].

Dermatoscopy is a noninvasive, widely used real-time diagnostic imaging technique, which renders magnification and translucency of the corneal layer, thus visualizing structures unseen with the naked eye. Dermatoscopy has been shown to improve diagnostic accuracy of pigmented and nonpigmented skin lesions. In the case of AKs, a sensitivity of 98.7% and specificity of 95.0% has been reported [8]. Similarly, for BCC a sensitivity of 95–98.6% and a diagnostic probability of as high as 99% has been reported [9, 10]. Additionally, even in clinically ambiguous cases where BCC and SCC have been inversely misdiagnosed, dermatoscopy yields an odds ratio of 2.9 for the correct diagnosis [11]. The latest updated version of dermatoscopic criteria associated with BCC consist of 12 features describing pigment structures (6), vessel morphology (2), surface characteristics (2), and background structures correlating with dermal fibrosis (2). The criteria can be present in different combinations showing the dermatoscopic variability of BCC [12]. More signs and features can be found in the literature but are less commonly used [13].

Both AK and BCC can cause high morbidity and treatment costs, especially if diagnosis and treatment is delayed thus allowing BCC to increase in size and possibly to a more aggressive subtype [14, 15] and AK to progress towards invasive SCC. Therefore, early diagnosis and management has a crucial role.

We report a case where the diagnosis of BCC was dermatoscopically possible after treatment of overlying AK.

Case Report

An 87-year-old fair-skinned (type II according to Fitzpatrick) female patient with no previous history of nonmelanoma skin cancer presented with multiple AKs on the facial skin. Lesions had been present for approximately 5 years. On clinical assessment, 20 AKs were identified, corresponding to 5.2 on the Actinic Keratosis Area and Severity Index [16] and 2 on the Actinic Keratosis Field Assessment Scale [17]. Before the therapy and during follow-up visits, all lesions were dermatoscopically assessed (polarized light contact dermoscopy) and digital images were taken with the FotoFinder Systems GmbH medicam 1000 device. One of the AKs on the left cheek showed a most inconclusive dermatoscopic picture and therefore was biopsied to exclude minimally invasive SCC. The histology showed AK grade III, parakeratosis, and lichenoid infiltration. No atypical mitoses were present. Treatment with 5% 5-fluoruracil cream was initiated according to the treatment guidelines and a follow-up visit was set after

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2 weeks. At that point, treatment had resulted in expected inflammatory response with erythema, erosions, mild crusting, some scaling and dermatoscopically seen peppering. A lesion on the nose that had previously shown a keratin mass and was considered as a hypertrophic AK (Fig. 1) now dermatoscopically consisted of two parts (Fig. 2). On the upper left side, a diffuse erythema, several plugged and targetoid hair follicles, and a whitish brown scale was present, while on the lower right side, short fine radially distributed linear and some branched vessels were visible. Therapy was continued for 1 more week and a follow-up visit was scheduled at 4 weeks following treatment. Then naked-eye examination showed slight depression and, apart from the previously pictured plugged hair follicles, short fine telangiectasia; some arborizing vessels with a small diameter and crystalline structures were visible on a translucent structureless background (Fig. 3). All of the present structures fulfill the dermatoscopic criteria for nonpigmented BCC. Cryodestruction of the newly diagnosed BCC was performed. At the posttreatment follow-up visit, the patient was clinically and dermatoscopically assessed by the treating physician as cleared of the BCC.

Discussion/Conclusion

This case report shows that treatment of AKs allows better clinical and dermatoscopic visualization of other nonpigmented skin tumors and therefore earlier diagnosis and treatment is possible. It can be considered as an additional treatment advantage apart from the well-known cosmetic benefit to the patient and possibly protection from the development of invasive SCC.

Historically the presence of collision lesions in dermatology has been reported by many authors, though AK and BCC was not among the most common combinations [18]. A more recent publication by Blum et al. [19] showed collision lesions between AK and BCC in 2 of 35 collision lesions with a BCC component. Side-by-side collision lesions between AK and BCC by some authors is considered a routine. Nevertheless, collision lesions in which AK precisely overlies BCC are a rarely reported entity. A study by Sambandan et al. [20] reported 8 cases in a 10-year period where biopsy of the superficial portion of the lesion was read as AK or SCC in situ, while deeper biopsy or surgery revealed infiltrative or nodular BCC. In reviewing the literature, we did not find another case where a BCC would have been dermatoscopically detected in a treatment follow-up of an overlying AK.

Some publications state possible progression from AK to BCC, though this idea is not generally accepted. For instance, Criscione et al. [21] observed that 36% of all primary BCCs in high-risk patients with previous nonmelanoma skin cancer arose in lesions that were previously clinically diagnosed as AKs. There is a possibility that the use of dermatoscopy would have increased the accuracy of clinical diagnosis as shown by Ryu et al. [11], who also concluded that BCC may be clinically misdiagnosed as SCC in the presence of scaling.

Three of the dermatoscopic criteria associated with BCC according to the latest updated criteria version were seen in this case – superficial fine telangiectasia, some arborizing vessels with small diameter, and crystalline structures [12]. An additional criterion, translucency, was also present. The named criteria are in consistency with the lesion being an initial BCC before the development of more common criteria according to the known correlations between dermatoscopic and morphological signs [22–27]. In particular, superficial fine telangiectasia and arborizing vessels with a small diameter have been associated with superficial BCCs [25, 26]. Translucency, also called semitranslucency, that can be seen both clinically and dermatoscopically as near-skin-tone colored, smooth, jellylike appearance has been suggested as a

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dermatoscopic sign of early BCCs measuring only 3–4 mm in diameter [22, 23]. Histologically translucency has been associated with verified nodular and morpheaform types of BCC [13]. Crystalline, also named chrysalis structures or shiny white streaks, represents collagenous stroma and fibrosis in the dermis [27]. In this case, we cannot exclude that crystalline structures could have appeared due to the treatment with 5% 5-fluoruracil cream. Absence of dermatoscopic pigment structures in this case is not unexpected, as they are more common in darker skin types [12, 28].

The treatment of AK in this case was indicated from the patient's perspective due to cosmetic burden and subjective symptom of slight prickling. From the physician's point of view, the patient was considered as a high-risk patient due to the high AK count (n = 20) on clinical examination. As shown by Green et al. [29], the relative risk of developing invasive SCC is increased by 5–6 times, if up to 20 AKs are present. The risk for developing a BCC is increased by up to 5 times if AK count exceeds 10 [7].

The patient was very content with the AK treatment result, although application site reactions were moderately acceptable.

The strength of this case report was the use of digital dermatoscopy on all AKs before, during and after the treatment and that the computer-aided method, which incorporates clinical and dermatoscopic pictures, allowed the same spot to be captured at every visit.

The limitation of this case was that a biopsy was not done on the newly developed BCC and histopathological subtype was not assessed. Dermatoscopic assessment of all AKs with treatment follow-up in a larger patient sample could identify more cases.

Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors have no conflicts of interest to declare.

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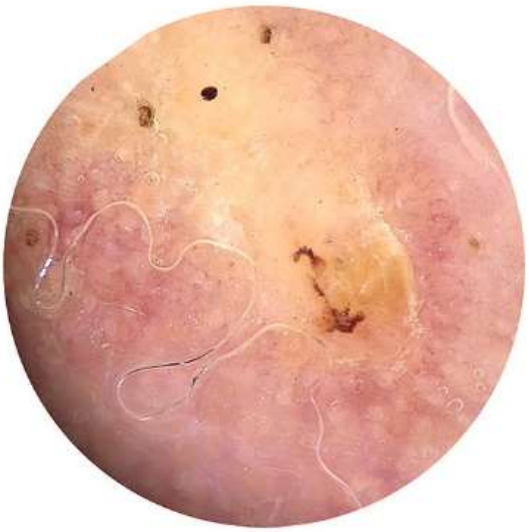

Fig. 1. Dermatoscopic image of a lesion on the nose clinically diagnosed as a hyperkeratotic actinic keratosis.

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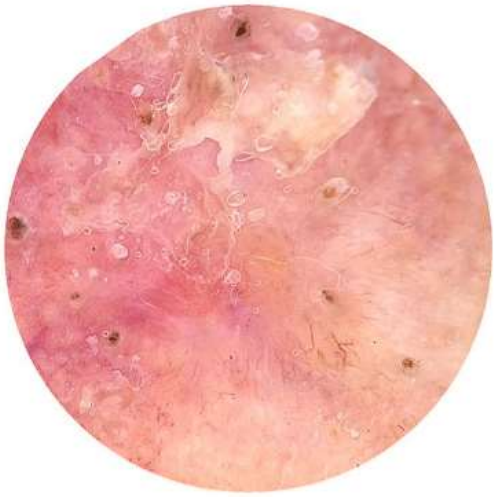

Fig. 2. Dermatoscopic image of the lesion after 2-week treatment with 5% 5-fluoruracil cream.

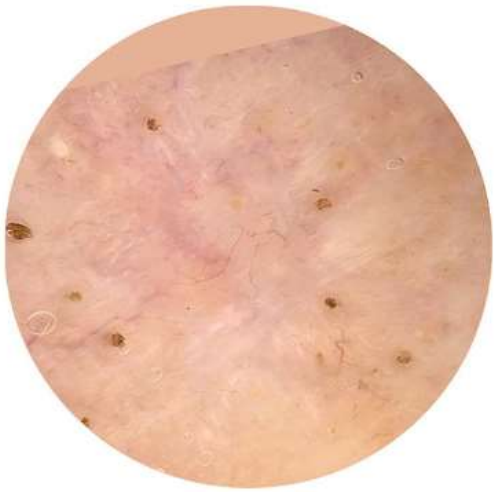

Fig. 3. Dermatoscopy of the lesion at a posttreatment follow-up visit. A translucent hue with short fine linear and branched vessels and shiny white streaks are seen.

Septītā publikācija

medicina

MDPI

Review

Prevalence, Discontinuation Rate, and Risk Factors for Severe Local Site Reactions with Topical Field Treatment Options for Actinic Keratosis of the Face and Scalp

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Abstract: Actinic keratoses (AKs) are common lesions on chronically sun damaged skin, which are morphologically characterized by lower third to full thickness atypia of epidermal keratinocytes. These lesions carry a risk of progression towards invasive squamous cell carcinoma (SCC); therefore, treatment of visible lesions and the field in case of field cancerization is recommended. Treatment of AK includes the destruction of atypical keratinocytes that clinically presents with various degrees of erythema, scaling, crusting, erosion, and other visible and subjective symptoms. Such inflammatory reactions may have an impact on the patient's social life and have shown to decrease compliance and adherence to therapy. Additionally, as various topical treatments have been proven to be effective in treating AK, tolerability of local site reactions (LSRs) might drive the decision for appropriate treatment in an individual scenario. Therefore, we aimed to review prevalence of severe LSRs among various topical treatments for AK. In addition, we summarized discontinuation rates due to LSRs and possible therapy-unrelated risk factors for the development of LSRs with increased severity.

Keywords: actinic keratosis; topical therapy; severe local site reactions; risk factors; review

1. Introduction

Actinic keratosis (AK) is one of the most common dermatological complaints in fair skinned individuals that represents the cumulative UV damage of epidermal keratinocytes. On clinical examination, AKs are variably thick and erythematous, poorly demarcated, and sometimes pigmented lesions on chronically sun-exposed skin [1]. Prevalence steadily increases with age and a recent Swiss study found that AK occurred in 25.3% of outpatients in general practice [2]. The clinical significance of AKs relies on the associated discomfort, cosmetic burden, and the possibility of progression to invasive squamous cell carcinoma (SCC). The traditional view considers that progression towards invasive SCC requires full thickness epidermal atypia, which is clinically usually characterized by thick and obvious lesions [3,4]. Nevertheless, a more recent study has shown that AK grade I, morphologically characterized by atypical keratinocytes in the lower third of the epidermis, is the most common type of AK overlying cutaneous invasive SCC [5]. This finding supports the understanding that all AKs require treatment. However, treatment is targeted towards the destruction of atypical keratinocytes, resulting in temporary inflammatory reactions with varying severity. Discussing the treatment-associated local site reactions (LSRs) before therapy may lead to delay in treatment initiation. During treatment, such reactions cause distress [6] and impact health-

related quality of life [7]. A study by Strohal et al. [8] showed that 19.4% of patients treated with imiquimod 5% cream, scheduled unplanned visits due to their concern of LSRs. Moreover, LSRs lead to nonadherence, thus possibly reducing treatment efficacy [9]. As topical therapy is easy to use and a highly effective treatment modality for AK, we aimed to systematically search the PubMed database to review prevalence of severe LSRs among various topical treatments for AK. In addition, we summarized discontinuation rates due to LSRs and possible therapy-unrelated risk factors for the development of LSRs with increased severity. We did not review studies involving special equipment as conventional photodynamic therapy. For this review, "LSRs" included visual parameters such as, but not limited to, erythema, oedema, and erosions, and patient-reported subjective symptoms, such as pain and itching.

2. Severe Local Site Reactions with Topical Field Treatment for Actinic Keratosis of the Face and Scalp

2.1. Prevalence of Severe Local Site Reactions

The prevalence of severe LSRs among various topical field treatment options is summarized in Table 1 [10–28].

Table 1. Comparison of local site reaction (LSR) severity among various field treatment modalities.

Author	Year of Publication	Treatment Modality	Number of Patients	Application Frequency	Overall Prevalence of LSR	Prevalence of Severe LSR	Prevalence of Systemic Symptoms
Sotiriou et al. [21]	2018	DL-PDT with MAL	46	Once	71.6%	0%	0%
Sotiriou et al. [11]	2017	DL-PDT with MAL	26	Once	69.2%	0%	0%
See et al. [22]	2017	DL-PDT with MAL	81	Once	44.4%* (erythema)	1.2%	0%
Rubel et al. [23]	2014	DL-PDT with MAL	100	Once	39.0%	0%	0%
Serra-Guillén et al. [24]	2017	Imiquimod 5%	65	Once daily for 12 consecutive days	100%	58.5%	20%
Rivers et al. [25]	2008	Imiquimod 5%	42	Three times per week for 4 weeks followed by 4 weeks of rest and second cycle if needed	95.2% * (erythema)	8.8% * (scabbing or crusting)	14% * (upper respiratory infection)
Stockfleth et al. [26]	2007	Imiquimod 5%	828	Three times per week for 4 weeks followed by 4 weeks of rest and second cycle if needed	NR	31.8% * (erythema)	6% * (headache)
Alomar et al. [27]	2007	Imiquimod 5%	129	Three times per week for 4 weeks followed by 4 weeks of rest and second cycle if needed	96.9% * (erythema)	31.0% * (erythema)	NR
Korman et al. [28]	2005	Imiquimod 5%	241	Three times per week for 16 weeks	98.3% * (erythema)	33.2% * (erythema)	NR
Lebwohl et al. [12]	2004	Imiquimod 5%	215	Twice per week for 16 weeks	97.2% * (erythema)	17.7% * (erythema)	NR
Swanson et al. [13]	2010	Imiquimod 3.75%	160	Once daily 2-week on/off/on cycle	Almost all	33.8%	NR

Hanke et al. [14]	2010	Imiquimod 3.75%	162	Once daily 3-week on/off/on cycle	Almost all	54.9%	8% * (Influenza-like illness)
Skroza et al. [15]	2017	Ingenol mebutate gel 0.015%	130	Once daily for 3 consecutive days	100%	17.7% * (edema)	0%
Jim On et al. [16]	2016	Ingenol mebutate gel 0.015%	274	Once daily for 3 consecutive days,	100%	24.5%	NR
Pflugfelder et al. [19]	2012	3% diclofenac in 2.5% hyaluronic acid gel	418	Twice daily for 3 or 6 months	NR	13.6%	NR
Stockfleth et al. [10]	2011	3% diclofenac in 2.5% hyaluronic acid gel	185	Twice daily for up to 12 weeks	62.7%	11.9% **	
Simon et al. [20]	2015	5-fluorouracil 0.5%/salicylic acid 10.0%	33	Once daily for up to 6 weeks	8.8% * (erythema)	18.2%	NR
Stockfleth et al. [10]	2011	5-fluorouracil 0.5%/salicylic acid 10.0%	187	Once daily for up to 12 weeks	92.0%	27.8% **	
Stough et al. [18]	2008	5-fluorouracil 0.5%	277	Once daily for up to 4 weeks	87.0%	19.1%	NR

NR—not reported; DL-PDT—daylight photodynamic therapy; MAL—methyl aminolevulinate; * Prevalence of the highest symptom reported; ** Combined data of severe symptoms.

Imiquimod. Among all topical therapies, the highest prevalence of severe LSRs was reported in studies with imiquimod 5% [24] and imiquimod 3.75% [14], with treatment regimens not commonly used. The suggested treatment regimen for imiquimod 5% is three times per week for four weeks followed by four weeks of rest and a second cycle if needed. Such a regimen was used in three of the included studies, and reported the highest values of 31.0% [27] and 31.8% [26] for prevalence of severe erythema and 8.8% prevalence of severe scabbing or crusting [25]. In the last study by Rivers et al. [25], severe erythema was noted in 5% of patients. Such less frequent prevalence is probably due to study methodology as patient assessment was performed at baseline and at week 8. Comparatively lower 25.2% prevalence of severe erythema and 33.8% prevalence of any severe LSR was reported by Swanson et al. [13] with imiquimod 3.75% in two identical studies randomized to placebo.

Ingenol mebutate. Studies with ingenol mebutate gel 0.015% (IngMeb) mostly use composite scores to assess severity of LSRs. Each of six LSR parameters—erythema, flaking/scaling, crusting, swelling, vesiculation/pustulation, and erosion/ulceration is graded from 0 to 4, giving a maximum composite score of 24 [15,29]. Jim On et al. [16] reported data of two multicenter, randomized, parallel-group, double-blind, vehicle-controlled studies, involving face and scalp and classified patients with a composite score of 12 or higher as having a severe LSR. Thus, their reported prevalence of severe LSRs at day 4 was 24.5%. Skroza et al. [15] included 130 patients and assessed LSRs at day 3. The reported mean total composite scores did not allow to assess overall prevalence of severe reactions, but they reported grade 4 swelling in 17.7% of patients. Additionally, a study by Ricci et al. [30] reported severe pain and itching in 20.5% of treatment cycles with IngMeb. This was not included in the chart as prevalence was calculated from treatment cycles, not patients.

5-fluorouracil. Since its approval in 1970, topical 5-fluorouracil (5-FU) has become a well-established treatment for AK and LSRs are expected [31,32]. Three formulations – 5-FU 5%, 5-FU 0.5% and 5-FU 0.5% in combination with a salicylic acid 10% solution (5-FU/SA) are commercially available. A prospective, open-label, multicenter study by Stough et al. [18] included 277 patients treated with once daily application of 5-FU 0.5% cream for up to 4 weeks. Interim results of the face and scalp showed that severe LSRs developed in 19.1% of patients. Two studies assessed efficacy and safety of 5-FU/SA. In the first by Stockfleth et al. [10], in a randomized, placebo-controlled, double-blind, multicenter trial conducted in Germany, low dose 5-FU/SA was compared with 3% diclofenac

in 2.5% hyaluronic acid gel (diclofenac HA). General disorders and administration-site conditions of severe intensity were reported in 27.8% of patients in the 5-FU/SA group and in 11.9% of patients in the diclofenac HA group. The second study by Simon et al. [20] compared 5-FU/SA with cryotherapy and included 33 patients in each treatment arm. Severe application site reactions were reported in six patients (18.2%) in 5-FU/SA arm and in one (3%) patient receiving cryotherapy.

Diclofenac HA. Pflugfelder et al. [19] reported 13.6%, which is the highest prevalence of severe LSRs in treatment with diclofenac HA. In a multicenter, randomized, open-label study with 418 included patients, they compared three-month vs. six-month treatment of actinic keratoses with diclofenac HA. Severe LSRs developed mainly in the first weeks of treatment and longer treatment only slightly increased the mean intensities of LSRs [19]. As already mentioned above, Stockfleth et al. [10] in a randomized, placebo-controlled, double-blind, multicenter trial conducted in Germany, reported general disorders and administration-site conditions of severe intensity in 11.9% of patients in diclofenac HA group.

Daylight photodynamic therapy. Only a single study reported a severe LSR in treatment with daylight photodynamic therapy (DL-PDT). This was a prospective, observational study conducted in Australia by See et al. [22], and they reported a single patient with severe post-treatment phototoxic reaction with erythema, pain, pruritus, and a skin burning sensation. This allowed a calculation of 1.2% of severe LSRs. Other studies on DL-PDT reported that none of the treated patients experienced severe LSRs [11,21,23].

2.2. Treatment Discontinuation Due to Local Site Reactions

In total, 14 articles reported treatment discontinuation rates due to LSRs with commonly used therapeutic regimens (Figure 1). The highest discontinuation rate of 13.6% was reported in a study by Pflugfelder et al. [19] that compared efficacy, tolerability, and quality of life of diclofenac HA used twice daily for three or six months. The study included 418 patients and eczematous reactions leading to discontinuation developed mainly in the first weeks of treatment. The second study with twice daily application of diclofenac HA was conducted by Stockfleth et al. [10] and reported a lower 4.9% discontinuation rate due to LSRs. Albeit, in this study, in the case of severe LSRs, application frequency was allowed to be reduced to once daily. The second highest rate of discontinuation due to LSRs was reported with 5-FU/SA by Simon et al. [20]. In the study evaluating efficacy, tolerability, and safety of low-dose 5-FU/SA topical solution vs. cryosurgery, they reported 9.1% discontinuation in the 5-FU/SA arm of 33 patients. As cryotherapy is a one-off treatment, it cannot be used as a true discontinuation comparator. Two other studies with 5-FU/SA and larger sample size reported 0.9% and 3.7% discontinuation rates [30,33]. Two studies reported discontinuation rates with low dose 5-FU cream. A study by Smith et al. [34] had 12 patients in the 5-FU 0.5% cream group, and the discontinuation of a single patient allowed a calculation of an 8.3% discontinuation rate. A study by Stough et al. [18] had 277 patients included and reported a discontinuation rate of 0.4%. A 5% 5-FU cream was used in one of the identified studies and included 50 patients. No discontinuations were reported [35]. Three articles showed discontinuation rates ranging from 0.5% to 3.2% with imiquimod 5% cream [25,27,36]. A discontinuation rate of up to 1.1% was reported in the studies with IngMeb [17,35,37]. This highest value was due to severe erosions in one of 88 included patients [37]. Treatment discontinuation due to LSR is not a concern for DL-PDT [11,21–23,38].

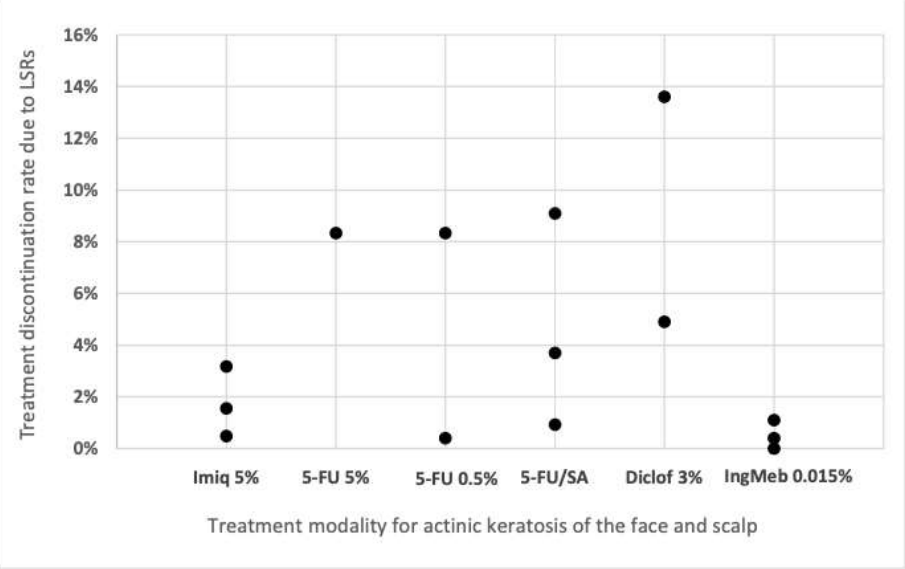

Figure 1. Reported rates of treatment discontinuation due to LSRs. Imiq 5%—imiquimod 5% cream; 5-FU 5%—5-fluorouracil 5% cream; 5-FU 0.5%—5-fluorouracil 0.5% cream; 5-FU/SA—5-fluorouracil in combination with salicylic acid 10% solution; Diclof 3%—3% diclofenac in 2.5% hyaluronic acid gel; IngMeb 0.015%—ingenol mebutate 0.015% gel.

2.3. Therapy-Unrelated Risk Factors for Development of Local Site Reactions with Increased Severity

Severity of LSRs depends on the administered active ingredient and application frequency. Additionally, several studies have implications on patient-associated and environmental risk factors. Primarily, light pigmentation is a risk factor for both AK and treatment-induced severity of LSRs [39]. A study with IngMeb by Ricci et al. [37] with a standard patient-applied regimen of once daily IngMeb for three consecutive days, showed that patients with a fair skin type (phototype I–II) had stronger LSRs at day 4 and more erosions than patients with phototype III–IV. Moreover, although DL-PDT generally does not cause severe LSRs, in a study by Galvão et al. [40], moderate erythema after two hours of outdoor exposure was seen only in an albino patient. Other proposed risk factors for greater severity of LSRs with IngMeb are: the female gender, an age below 70 years, and Korean patients [41]. This last observation was suggested to be related to the difference of race and skin thickness [42]. The importance of environmental factors has been suggested in a study by Fargnoli et al. conducted in Italy from September to October. In particular, high outdoor temperature was associated with severity of LSRs and treatment efficacy of DL-PDT [38].

3. Conclusions

Local site reactions of severe intensity seem to be extremely common among topical therapies for AK, especially with imiquimod. The only therapeutic modality with low prevalence of severe LSRs is DL-PDT. Treatment discontinuation due to LSRs is also common, although the highest prevalence of treatment discontinuation due to LSRs is reported in studies with the longest treatment regimens, as with diclofenac, and not in studies reporting the highest prevalence rates of severe LSRs. Several patient-associated risk factors for the development of severe LSRs have been identified in studies with DL-PDT and IngMeb. Nevertheless, to have better evidence of individual risk for severe LSRs, further studies could identify more risk factors, and include other therapies.

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Ētikas komiteju atļaujas

Veidlapa Nr. E-9 (2)

RSU ĒTIKAS KOMITEJAS LĒMUMS NR. 50 / 22.02.2018.

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Pieteikuma iesniedzējs: **Alise Balcere**
Medicīnas fakultāte

Pētījuma nosaukums: "Aktīnisko keratožu klīnisko, dermatoskopisko un morfoloģisko parametru raksturojums un to korelācija ar veidojumu prognozi"

Iesniegšanas datums: 22.02.2018.

Pētījuma protokols: Izskatot augstāk minētā pētījuma pieteikuma materiālus (protokolu) ir redzams, ka pētījuma mērķis tiek sasniegts veicot ar pacientiem klīniski-analītisku pētījumu (audu paraugu ņemšanu), dermatoskopisku fotodokumentēšanu un pacientu/dalībnieku aptauju, iegūto datu apstrādi un analīzi, kā arī izsakot priekšlikumus. Personu (pacientu, dalībnieku) datu aizsardzība, informēta brīvprātīga piedalīšanās un konfidencialitāte ir ievērota un nodrošināta. Līdz ar to pieteikums atbilst pētījuma ētikas prasībām.

Izskaidrošanas formulārs: ir

Piekrišana piedalīties pētījumā: ir

Komitejas lēmums: **piekrist** pētījumam

Komitejas priekšsēdētājs Olafs Brūvers Tituls: Dr. miss., prof.

Paraksts

RĪGAS STRADIŅA UNIVERSITĀTE ĒTIKAS KOMITEJA

Ētikas komitejas sēdes datums: 22.02.2018.

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ar Rīgas Stradiņa universitātes rektora
2018. gada 26. septembra rīkojumu Nr. 5-1/238/2018

Rīgas Stradiņa universitātes
Pētījumu ētikas komitejas
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03.12.2021 22-2/548/2021

	Komitejas sastāvs	Kvalifikācija	Nodarbošanās
1	Profesors Jānis Vētra	Dr.habil. med.	Morfoloģijas katedra
2	Asoc. Prof. Zanda Daneberga	Dr.med.	OI Molekulārās ģenētikas laboratorijas vadītāja
3	Asoc. Prof. Anita Vētra	Dr.med.	Rehabilitācijas katedras vadītāja
4	Profesore Ingrīda Čēma	Dr.habil. med.	Mutes medicīnas katedras vadītāja
5	Docente Anna Junga	Dr.med.	Morfoloģijas laboratorijas vadītāja
6	Pētniece p.i. Karina Palkova	Ph.D.	Advokāte, Doktora studiju programmas vadītāja
7	Marina Siņkovska		Datu drošības un pārvaldības daļas vadītāja

Pieteikuma iesniedzējs/i: **Alise Balcere**

Pētījuma / pētnieciskā darba nosaukums: Klīnisko un dermatoskopisko kritēriju salīdzinājums augsta progresijas riska sejas ādas plakanšūnu karcinomu in situ identificēšanai

Pētījumu ētikas komitejas sēdes datums: 25.11.2021

Pētījuma protokols: Izskatot augstāk minētā pētījuma pieteikuma materiālus (protokolu) ir redzams, ka pētījuma mērķis tiek sasniegts iekļaujot pacientus, klīniskā pētījuma kontroles grupā, kur bez kāda apdraudējuma veselībai, drošībai un dzīvībai, ņems atlieku audus, kas iegūti, veicot ķirurģiskās brūces plastiku ar rotācijas lēveri (tiek saglabāts princips, ka pacientam netiek veikta lielāka iejaukšanās - ekscidēto audu lielums nav lielāks, kā gadījumā, ja pacients nepiedalītos pētījumā), kā arī audu materiālus, kas iegūti atkāpjoties no jebkura audzēja, ja klīniski nav vērojama aktīnisko keratožu lauka kanceroģenēze. Tiks veikta iegūto datu apstrāde un analīze. Personu (pacientu) informēta brīvprātīga piekrišana piedalīties, personu iegūto datu apstrāde un aizsardzība, to pielietošana, glabāšana, anonimitāte un konfidencialitāte ir nodrošināta. Līdz ar to pieteikums atbilst pētījuma ētikas prasībām.

Komitejas lēmums: **Piekrist** pētījumam.

Komitejas priekšsēdētājs Jānis Vētra Tituls: Dr.habil. med., profesors.

ŠIS DOKUMENTS IR ELEKTRONISKI PARAKSTĪTS AR DROŠU ELEKTRONISKO PARAKSTU UN SATUR LAIKA ZĪMOGU

K. Ķauķe
Tālrunis: 26691306

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RĪGAS AUSTRUMU KLĪNISKĀ UNIVERSITĀTES SLIMNĪCA

SIA Rīgas Austrumu klīniskā universitātes slimnīca
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APSTIPRINĀTS
ar SIA „Rīgas Austrumu klīniskā universitātes slimnīca" valdes 2019. gada 22. janvāra lēmumu
Nr. V1/01-01/19/31

Rīgā

RSU studentei
Alisei Balcerei

2021.gada 10. augustā
Nr. *ZD/08-06/01-21/168*

ATĻAUJA AKADĒMISKĀ PĒTĪJUMA VEIKŠANAI

Zinātnes daļa ir izskatījusi Jūsu iesniegto akadēmiskā pētījuma „***Klīnisko un dermatoskopisko kritēriju salīdzinājums augsta progresijas riska sejas ādas plakanšūnu karcinomu in situ identificēšanai***" dokumentāciju, kas reģistrēta Zinātnes daļā ar numuru **AP-99/21**, kas apstiprina akadēmiskā pētījuma veikšanu SIA „Rīgas Austrumu klīniskā universitātes slimnīca" (turpmāk – Iestāde) stacionāra "Latvijas Onkoloģijas centrs" Onkoķirurģijas klīnikā, vadītājs Armands Sīviņš un Patoloģijas centrā, vadītājs Valdis Miķelsons.

Atbildīgais par pētniecības norisi Iestādē ir Ingrīda Čēma.

Zinātnes daļā iesniegti un izskatīti:

1. Pieteikums par akadēmiskā pētījuma AP-99/21 veikšanu,
2. Pētījuma protokols ar pielikumu,
3. Alises Balceres konfidencialitātes apliecinājums,
4. Ētikas komitejas atzinums, izsniegts 2021.gada 8.jūlijā.

Prospektīvā pētījumā, iegūstot pacientu rakstisku piekrišanu, 40 Ambulatorās daļas aktīniskās keratozes pacientiem tiks novērtēta klīniskā aina un iegūts biopsijas materiāls. Pētījuma veikšanai paredzētie izlietojamie materiāli tiks iegādāti par RSU doktorantūras studiju granta un privātiem līdzekļiem. Imūnhistoķīmisko krāsojumu veikšanai jāslēdz līgums ar Patoloģijas centru.

Pētnieku pienākums ir izpildīt 2020.gada 9.jūlija "Infekciju izplatības ierobežošanas pasākumu kārtība SIA "Rīgas Austrumu klīniskajā universitātes slimnīca" prasības.

Atļauja derīga līdz 2022.gada 31. augustam.

Dr.med. Daiga Šantare

(paraksts)

Speciāliste akadēmisko pētījumu jautājumos
Zinātnes daļa
Šantare, 67303179

APSTIPRINĀTS
ar SIA „Rīgas Austrumu klīniskā universitātes slimnīca" valdes 2019. gada 22. janvāra lēmumu
Nr. V1/01-01/19/31

aslimnīca
RĪGAS AUSTRUMU KLĪNISKĀ UNIVERSITĀTES SLIMNĪCA

SIA Rīgas Austrumu klīniskā universitātes slimnīca
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Rīgā

RSU studentei
Alisei Balcerei

2021.gada 7. decembrī
Nr. ZD/08-07/01-21/18

IZMAIŅAS ATĻAUJĀ AKADĒMISKĀ PĒTĪJUMA VEIKŠANAI

Zinātnes daļa ir izskatījusi Jūsu iesniegto akadēmiskā pētījuma „***Klīnisko un dermatoskopisko kritēriju salīdzinājums augsta progresijas riska sejas ādas plakanšūnu karcinomu in situ identificēšanai***" dokumentāciju, kas reģistrēta Zinātnes daļā ar numuru **AP-99/21**, kas apstiprina akadēmiskā pētījuma veikšanu SIA „Rīgas Austrumu klīniskā universitātes slimnīca" (turpmāk – Iestāde) stacionāra "Latvijas Onkoloģijas centrs" Onkoķirurģijas klīnikā, vadītājs Armands Sīviņš un Patoloģijas centrā, vadītājs Valdis Miķelsons.

Atbildīgais par pētnieciības norisi Iestādē ir Ingrīda Čēma.

Zinātnes daļā iesniegti un izskatīti:

1. Pieteikums par akadēmiskā pētījuma AP-99/21 veikšanu,
2. Pētījuma protokols ar pielikumu,
3. Alises Balceres, Egila Koreņeva un Gunāra Lauska konfidencialitātes apliecinājumi,
4. Ētikas komitejas atzinums, izsniegts 2021.gada 8.jūlijā.
5. Ētikas komitejas atzinums, izsniegts 2021.gada 3.decembrī.
6. A.Balceres 2021.gada 27.oktobra iesniegums par izmaiņām.

Prospektīvā pētījumā, iegūstot pacientu rakstisku piekrišanu, 40 Ambulatorās daļas aktīniskās keratozes pacientiem tiks novērtēta klīniskā aina un iegūts biopsijas materiāls. Pētījuma veikšanai paredzētie izlietojamie materiāli tiks iegādāti par RSU doktorantūras studiju granta un privātiem līdzekļiem. Imūnhistoķīmisko krāsojumu veikšanai jāslēdz līgums ar Patoloģijas centru.

Pētnieku pienākums ir izpildīt 2020.gada 9.jūlija "Infekciju izplatības ierobežošanas pasākumu kārtība SIA "Rīgas Austrumu klīniskajā universitātes slimnīca" prasības.

Pamatojoties uz A Balceres 2021.gada 27.oktobra iesniegumu, kā pētījuma izpildītāji pievienoti E.Korņevs un G.Lausks, paplašināts iekļaujamo pacientu skaits, precizēts iegūstamais bioloģiskais materiāls. Izmaiņas saskaņojusi ētikas komiteja.

Atļauja derīga līdz 2022.gada 31. augustam.

Dr.med. Daiga Šantare

(paraksts)

Speciāliste akadēmisko pētījumu jautājumos
Zinātnes daļa
Šantare, 67303179

Pacientu piekrišanas formas

Informētās piekrišanas veidlapa dalībai biomedicīnas pētījumā

Cienītā kundze!
Godātais kungs!

Mēs uzaicinām Jūs piedalīties pētījumā ***Klīnisko un dermatoskopisko kritēriju salīdzinājums augsta progresijas riska sejas ādas plakanšūnu karcinomu in situ identificēšanai***, ko veic sertificēts dermatologs, venerologs un RSU Doktorantūras nodaļas doktorante Alise Balcere. Vēlamies Jūs iepazīstināt ar pētījuma mērķi, norisi un saturu. Pirms šī dokumenta parakstīšanas rūpīgi izlasiet visu informāciju! Pirms dokumenta parakstīšanas Jums ir tiesības uzdot jautājumus par pētījumu un saņemt uz tiem atbildes.

Pētījuma mērķis: Noteikt, vai pacientiem ar multiplām aktīniskajām keratozēm ir atsevišķi klīniski un/vai dermatoskopiski atšķirīgi veidojumi un salīdzināt klīniskos, dermatoskopiskos un morfoloģiskos (no ādas biopsijas iegūtos) datus, lai identificētu, vai klīniskā un/vai dermatoskopiskā atipija saistās ar augstāku morfoloģiski noteiktu veidojumu progresijas risku.

Pētījuma norise:
Pētījuma ietvaros pacienti tiks aptaujāti atbilstoši pētījuma protokolam, kas ietver jautājumus par aktīniskajām keratozēm, tai skaitā to ilgumu un saņemto ārstēšanu. Papildus tiks klīniski novērtēta sejas āda, tai skaitā aktīnisko keratožu smaguma pakāpe atbilstoši klīniskajām skalām AKASI un AK-FAS, kas izvērtē kopējo slimības smaguma pakāpi atbilstoši veidojumu izplatībai, apsārtumam, biezumam un saules bojājuma smagumam, un tiks izvērtēta katra veidojuma klīniskā un dermatoskopiskā aina, tā identificējot atšķirīgus veidojumus, kas atbilstoši pētījuma hipotēzei ir ar augstāku risku progresēt par invazīvu plakanšūnu karcinomu. Lai šo risku izvērtētu, ir paredzēts paņemt 4 mm perforācijas biopsiju veidojuma patohistoloģiskajam izvērtējumam. Tā ir invazīva procedūra, kas apstiprina diagnozi un pirms kuras paredzētajā vietā tiks infiltrēta 2% lidokaīna anestēzija, savukārt procedūras rezultātā radies audu defekts tiks slēgts atbilstoši ādas līnijām ar šuvēm, kas nodrošinās hemostāzi un straujāku dzīšanu. Jebkura pacientam būtiska informācija, kas atklāsies patohistoloģiskajā izmeklēšanā, piemēram, cita diagnoze, kā bazālo šūnu karcinoma, tiks pacientam paziņota, ja viņš būs devis piekrišanu, zemāk norādot savu tālruņa numuru. Lai nodrošinātu klīnisko un dermatoskopisko pazīmju atkārtotu izvērtējumu, pirms procedūras tiks veikta fotodokumentēšana, kas var tikt izmantota zinātnisku publikāciju gatavošanai ar mērķi atspoguļot pētījuma rezultātus un izglītot medicīnas profesionāļus.

Ieguvumi:
Pētījuma ietvaros pacientam tiks piedāvātas konsultācijas pie pētījuma autores sertificēta dermatologa, venerologa dr. Alises Balceres aktīnisko keratožu terapijas nozīmēšanai un aplikācijas vietu reakciju smaguma pakāpes monitorēšanai, kā arī veiktā ādas biopsija kalpos par apstiprinājumu klīniskajai diagnozei, izslēgs minimāli invazīvas plakanšunu karcinomas esamību un diferenciāldiagnozes. Pētījuma rezultāti ļaus ārstiem precīzāk identificēt veidojumus ar augstāku malignitātes risku, kas ļautu savlaicīgi piemērot adekvātu terapiju konkrētajiem veidojumiem arī gadījumos, kad lauka terapija ir atliekama.

Iespējamie riski:
Pētījuma ietvaros veiktā ādas biopsijas procedūra saistās ar nelielu diskomfortu anestēzijas ievades laikā un iespējams bojājuma dzīšanas laikā, kā arī minimālu ādas infekcijas attīstības risku. Pēc sadzīšanas ir iespējama rētas attīstība, kaut arī parasti tā ir minimāla un nav pamanāma. Pārējā pētījuma materiāla iegūšana (aptauja, klīniskā un dermatoskopiskā novērtēšana un fotodokumentēšana) nerada papildus risku vai kaitējumu pacienta veselībai, bet var būt saistīta ar emocionālu diskomfortu.

Konfidencialitāte un datu drošība:
Pētījumā iekļautie pacientu dati, klīniskie un dermatoskopiskie attēli un patohistoloģiskās izmeklēšanas rezultāti tiks kodēti un apstrādāti anonīmā veidā. Klīniskie attēli ir būtiski atipisko veidojumu klīnisko pazīmju identificēšanai, un, tos izmantojot mācību vai publikāciju nolūkā, tiks mazināta pacienta atpazīstamība, attēla centrā atstājot konkrēto veidojumu vai veidojumus, ja tie ir vairāki, kā arī daļu attēla aizklājot. Ja pētījuma dalībnieks pārtrauc dalību pētījumā, tad viņa pētījumā iekļautā informācija tiks dzēsta, bet biopsijas materiāls atdots pacientam vai iznīcināts, atbilstoši viņa izvēlei. Dati var tikt atkodēti, ja biopsijas materiālā tiek konstatētas pārmaiņas, piemēram, invazīvi ādas audzēji, kas nav pilnībā izņemti perforācijas biopsijas laikā un pacients ir devis piekrišanu šādos gadījumos viņu sakontaktēt pa tālruņa numuru _____.

Brīvprātīga piedalīšanās:
Piedalīšanās šajā pētījumā ir brīvprātīga. Jums ir tiesības atteikties piedalīties pētījumā vai pārtraukt dalību pētījumā jebkurā laikā. Jūsu atteikšanās piedalīties pētījumā vai dalības pārtraukšana neradīs nekādu nevēlamu ietekmi uz Jums sniegtās veselības aprūpes kvalitāti.
Ja jums ir jebkādi jautājumi par šo pētījumu, lūdzu, sazinieties ar dr. Alisi Balceri, RSU Dermatoloģijas un veneroloģijas katedra, Rīgā, Baznīcas ielā 18, alise.balcere@gmail.com, 26467126. Šis pētījums ir apstiprināts Rīgas Stradiņa universitātes Pētījumu ētikas komitejā: RSU centrālā ēka, Rīga, Dzirciema iela 16.

Šis dokuments ir sastādīts divos eksemplāros, no kuriem viens atrodas pie pētījuma veicēja, bet otrs – pie pētāmās personas.

Es ar savu parakstu apliecinu, ka

1) esmu iepazinies/-usies ar šī dokumenta saturu;
2) uz maniem jautājumiem ir sniegtas atbildes,
3) es piekrītu piedalīties šajā pētījumā,
4) es saprotu, ka mana dalība šajā pētījumā ir brīvprātīga, un atteikšanās piedalīties pētījumā vai dalības pārtraukšana neizraisīs nekādas nelabvēlīgas sekas;
5) piekrītu, ka šī pētījuma laikā, atbilstoši tiesiskā regulējuma prasībām, tiek savākti, uzglabāti un apstrādāti mani personas dati, tai skaitā medicīnisko izmeklējumu rezultāti.

_____ _____
Vārds, uzvārds Datums Paraksts

Pētnieks:

_____ _____
Vārds, uzvārds Datums Paraksts

Informētās piekrišanas formas sākotnējā versija

Informētas pacienta piekrišanas dalībai pētījumā veidlapa

Es, _____, personas kods: _____,

/Vārds, Uzvārds/

□ Piekrītu, ka pētījuma ietvaros tiek apkopoti mani demogrāfiskie un ar slimības vēsturi saistītie dati, kā arī veikts manas ādas klīniskais novērtējums, uzņemti klīniskie un atsevišķu veidojumu dermatoskopiskie fotoattēli un paņemts ādas biopsijas paraugs.

□ Piekrītu, ka pētījuma ietvaros tiek apstrādāti mani personas dati, kas izriet no demogrāfijas, ādas klīniskā novērtējuma, ādas klīniskajiem un atsevišķu veidojumu dermatoskopiskajiem fotoattēliem un ādas biopsijas parauga.

Esmu informēts, ka paņemtais biopsijas materiāls sākotnēji tiks histopatoloģiski izmeklēts, kas ļaus precizēt diagnozi. Ja histopatoloģiskās izmeklēšanas laikā atklāsies manai veselībai būtiska informācija, es piekrītu, ka ar mani sazināsies pa tālruni: _____.

Es apzinos, ka pētījuma rezultāti var tikt demonstrēti konferencēs un semināros un publicēti profesionālajos žurnālos u.c. informācijas resursos ar mērķi atspoguļot pētījuma rezultātus un izglītot medicīnas nozares profesionāļus. Es saprotu, ka jebkura mani identificējoša informācija būs konfidenciāla, ka mani klīniskie un dermatoskopiskie attēli un ādas biopsijas paraugi būs kodēti un ka klīniskajos attēlos būs redzams slimības skartais ādas rajons, bet aizklāti citi rajoni, tā novēršot manu atpazīstamību. Es apzinos, ka es jebkurā brīdī bez paskaidrojumiem varu pārtraukt dalību pētījumā, zinot, ka tas neietekmēs manu turpmāko ārstēšanos. Zinu, ka šādā gadījumā mani klīniskie un dermatoskopiskie attēli, nodotie ādas biopsijas paraugi, veselības stāvokļa apraksts un jebkura mani identificējoša informācija tiks iznīcināta. Es piekrītu, ka manus ādas biopsijas paraugus izmanto slimības diagnostikā, izvērtējot arī imūnhistoķīmiski. Es saprotu, ka ādas biopsijas izmeklēšana nerada risku manai veselībai, kas man ir izskaidrots.

Esmu sapratis informāciju, kas man ir dota par šo pētījumu.

_____

/Paraksts/

Rīgā, 20____. gada ____. _____

Infromētās piekrišanas forma kontroles biopsiju iegūšanai

Cienītā kundze!
Godātais kungs!

Mēs uzaicinām Jūs piedalīties pētījumā *Klīnisko un dermatoskopisko kritēriju salīdzinājums augsta progresijas riska sejas ādas plakanšūnu karcinomu in situ identificēšanai*, ko veic *dr. Alise Balcere* un kas tiek finansēts no doktorantūras studiju granta līdzekļiem. Vēlamies Jūs iepazīstināt ar pētījuma mērķi, norisi un saturu. Pirms šī dokumenta parakstīšanas rūpīgi izlasiet visu informāciju! Pirms dokumenta parakstīšanas Jums ir tiesības uzdot jautājumus par pētījumu un saņemt uz tiem atbildes.

Pētījuma mērķis:
Identificēt būtiskākos klīniskos un dermatoskopiskos kritērijus, kas ļauj laicīgi atklāt augsta riska aktīniskās jeb saules keratozes, kas ir bieži veidojumi sejas ādā gados vecākiem pacientiem un var progresēt par invazīvu plakanšūnu karcinomu. Lai pārliecinātos par klīnisko un dermatoskopisko kritēriju nozīmi, tiks veikta mikroskopiskā jeb histopatoloģiskā izmeklēšana, kas ir zelta standarts ikvienas ādas slimības diagnostikā. Tai pat laikā, lai atšķirtu būtiskākās ādas veidojumu mikroskopiskās pazīmes, tās ir jāsalīdzina ar klīniski veselas ādas mikroskopiskajām īpašībām. Šādi izdarot secinājumus par klīniskajiem un dermatoskopiskajiem kritērijiem būs pamats izstrādāt vadlīnijas ārstiem agrīnākai audzēju diagnostikai.

Pētījuma norise:
Pētījums tiks veikts Latvijas Onkoloģijas centra ambulatorajā daļā no 2021. gada rudens līdz 2022. gada pavasarim. Pētījumā paredzēts pēc informētas piekrišanas iegūšanas veikt Jums paredzēto ķirurģisko procedūru atbilstoši visiem ārstēšanas standartiem, gluži, kā tad, ja Jūs pētījumā nepiedalītos. Pēc veidojuma ekscīzijas no izņemto audu stūra paredzēts atdalīt dažus milimetrus lielu klīniski veselas ādas fragmentu. Šis fragments, kā to paredz medicīniskā prakse, tiek nogādāts patoloģijas laboratorijā kopā ar izoperēto ādas veidojumu, lai pārliecinātos, ka arī tas nesatur diagnozes izplatībai būtisku operētā veidojuma daļu. Ja, tas patiesi ir klīniski un morfoloģiski veselas ādas fragments, tad šī pētījuma ietvaros tam papildus tiks veikti imūnhistoķīmiskie izmeklējumi, lai izdarītu secinājumus par veselas ādas īpašībām. Papildus datu analīzē tiks iekļauta informācija par Jūsu dzimumu, vecumu un operētā veidojuma diagnozi.

Ieguvumi:
Klīnisko un dermatoskopisko kritēriju izstrāde ļaus laicīgi un neinvazīvi diagnosticēt tās atsevišķās aktīniskās keratozes, kas ir ar augstāku risku attīstīties par invazīviem audzējiem.

Iespējamie riski:
Pētījuma ietvaros pacientam netiek veiktas papildus manipulācijas, kas varētu radīt risku.

Konfidencialitāte un datu drošība:
Personas datu apstrāde notiks atbilstoši "Fizisko personu datu apstrādes likuma" prasībām. Lai apstiprinātu dalību pētījumā, dalībniekam ir jāparaksta informētas piekrišanas veidlapa, norādot savus personas datus – vārdu, uzvārdu un parakstu. Šīs veidlapas glabāsies pie prof. Ingrīdas Čēmas un/vai dr. Alises Balceres un tiks uzskatītas par ārsta noslēpumu, kas netiks izpausts. Ja histoloģiski tiks apstiprināts, ka audu paraugs atbilst pētījuma prasībām, tad tas tiks izņemts kā bloks ar šifrētu numuru. Ja dalībnieks izvēlas pārtraukt dalību pētījumā, viņa audu materiāls tiks atgriezts laboratorijā, ja tas būs iespējams (nebūs jau veikti visi krāsojumi), savukārt, iegūtie rezultāti dzēsti. Pētījuma rezultāti tiks publicēti tikai apkopotā veidā. Ja Jums ir jautājumi vai sūdzības par Jūsu personas datu apstrādi un uzglabāšanu šajā pētījumā, Jums jāsazinās ar dr. Alisi Balceri, tel. 26467126, e-pasts: dr.abalcere@gmail.com.

Brīvprātīga piedalīšanās:
Piedalīšanās šajā pētījumā ir brīvprātīga. Jums ir tiesības atteikties piedalīties pētījumā vai pārtraukt dalību pētījumā jebkurā laikā. Jūsu atteikšanās piedalīties pētījumā vai dalības pārtraukšana neradīs nekādu nevēlamu ietekmi uz Jums sniegtās veselības aprūpes kvalitāti. Mēs informēsim Jūs par visiem būtiskajiem jautājumiem par šo pētījumu, kas var ietekmēt Jūsu vēlmi turpināt dalību šajā pētījumā.

Ja jums ir jebkādi jautājumi par šo pētījumu, lūdzu, sazinieties ar dr. Alisi Balceri, adrese: Rīgas Stradiņa universitātes Dermatoloģijas un veneroloģijas katedra, Baznīcas iela 18, Rīga; e-pasts: dr.abalcere@gmail.com.

Šis dokuments ir sastādīts divos eksemplāros, no kuriem viens atrodas pie pētījuma veicēja, bet otrs – pie pētāmās personas.

Es ar savu parakstu apliecinu, ka

1) esmu iepazinies/-usies ar šī dokumenta saturu;
2) uz maniem jautājumiem ir sniegtas atbildes,
3) es piekrītu piedalīties šajā pētījumā,
4) es saprotu, ka mana dalība šajā pētījumā ir brīvprātīga, un atteikšanās piedalīties pētījumā vai dalības pārtraukšana neizraisīs nekādas nelabvēlīgas sekas;
5) piekrītu, ka šī pētījuma laikā, atbilstoši tiesiskā regulējuma prasībām, tiek savākti, uzglabāti un apstrādāti mani personas dati, tai skaitā medicīnisko izmeklējumu rezultāti.

_____	_____	
Vārds, uzvārds	Datums	Paraksts

Pētnieks:

_____	_____	
Vārds, uzvārds	Datums	Paraksts