ORIGINAL REPORT

Clinical Risk Factors for Cutaneous Squamous Cell Carcinoma in Patients with Actinic Keratosis or Cutaneous Squamous Cell Carcinoma in Situ: A Retrospective Double-cohort Study

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Actinic keratosis and cutaneous squamous cell carcinoma *in situ* are precancerous forms of cutaneous squamous cell carcinoma. In this single-centre retrospective study, patients with histopathologically confirmed actinic keratosis (*n* = 121) or cutaneous squamous cell carcinoma *in situ* (*n* = 99) as their initial keratinocyte-derived lesion were compared and evaluated with regard to development of cutaneous squamous cell carcinoma during a 5-year observation period. Patients with severely dysplastic actinic keratosis or cutaneous squamous cell carcinoma *in situ* as their initial lesion developed cutaneous squamous cell carcinoma more rapidly than patients with actinic keratosis with mild or moderate dysplasia. With either actinic keratosis or cutaneous squamous cell carcinoma *in situ* as an initial lesion, advanced age, male sex, comorbidity with basal cell carcinoma, and immunosuppressive medication were associated with elevated risk of cutaneous squamous cell carcinoma development. Regarding solely patient with actinic keratosis as their initial lesion male sex, advanced age, immunosuppressive medication, location of the initial lesion, and degree of dysplasia were associated with the risk of cutaneous squamous cell carcinoma. Among patients with cutaneous squamous cell carcinoma *in situ* as their initial lesion, only aspirin usage was associated with increased risk of cutaneous squamous cell carcinoma. This study indicates that, among the vast and increasing population of patients with cutaneous squamous cell carcinoma precursors, male patients with immunosuppressive medication who develop basal cell carcinoma should be regarded as at heightened risk of cutaneous squamous cell carcinoma development and warrant closer surveillance.

Key words: actinic keratosis; cancer; cutaneous squamous cell carcinoma; cutaneous squamous cell carcinoma *in situ*; keratinocyte carcinoma

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SIGNIFICANCE

In this study risk factors for the development of cutaneous squamous cell carcinoma in patients with previously diagnosed precancerous skin lesions, actinic keratosis, or cutaneous squamous cell carcinoma *in situ* were investigated. Advanced age, male sex, comorbidity with basal cell carcinoma, and immunosuppressive medication were identified as risk factors for the development of cutaneous squamous cell carcinoma in patients with cutaneous squamous cell carcinoma *in situ* or actinic keratosis as their initial lesion. Patients with actinic keratosis with high-grade dysplasia or cutaneous squamous cell carcinoma *in situ* as their initial lesion develop cutaneous squamous cell carcinoma faster than patients with actinic keratosis with mild or moderate dysplasia. The results of this study provide evidence for these acknowledged risk factors and emphasize that within the vast population of patients with premalignant lesions these features prompt closer surveillance. The elevated risk of cutaneous squamous cell carcinoma development in cutaneous squamous cell carcinoma *in situ* patients with aspirin usage warrants further studies.

Cutaneous squamous cell carcinoma (cSCC) is the second most common cancer of the skin (1, 2). The incidence of this keratinocyte carcinoma is increasing and 2–5% of cSCCs are metastatic, which is associated with a poor prognosis (3, 4). Actinic keratosis (AK) and cutaneous squamous cell carcinoma *in situ* (cSCCIS), i.e., Bowen's disease, are ultraviolet (UV)-induced preinvasive cutaneous lesions, in which keratinocyte dysplasia is limited to the epidermis (5). AK is cytologically indistinguishable from cSCCIS and can be considered as a precancerous lesion or as *in situ* SCC that can transform into invasive cSCC (6, 7). AK represents the most common dermatological diagnosis in patients over 45 years of age in the United States and the incidence of AK in a British population over 60 years of age is 149 per 1,000 person-years (8, 9). The prevalence of AK is 34% in British white individuals over 70 years and up to 60% in an Australian over 40-year-old population (10,11). The incidence and prevalence of AK is increasing in general and increases significantly with age, reflecting the effect of UV-radiation exposure (12).

AK typically presents clinically as erythematous, scaly, and rough patch on sun-exposed skin (13, 14). Histologically AKs can be classified as low- (AK I), mode-

rate- (AK II), or high-grade dysplastic (AK III) based on the extent of atypical keratinocytes from dysplasia confined to the most basal third of the epidermis (AK I) to dysplasia that encompasses all thirds of the epidermis (AK III) (13). cSCCIS presents histologically with full epidermal thickness dysplasia and has traditionally been considered as the intermediate step in the progression of AK to invasive cSCC (15). There are distinct differences between AK III and Bowen's disease, although AK III is also considered to be synonymous with cSCCIS (16, 17).

AK can persist, regress, or progress into cSCC (7). Progression of AK to cSCC takes place via the classic or the differentiated pathway (18). In the classic pathway the extent of dysplasia progressively reaches the full epidermis before invading through basement membrane (18). In the differentiated pathway, the invasion takes place directly from the AK I, in which atypical keratinocytes are confined to the basal third of the epidermis (18). It has been reported that the majority of AK to cSCC progression is thought to take place via the differentiated pathway (18).

The progression of AK to cSCC involves alterations in the genome and tumour microenvironment (19, 20). The main risk factor for the development of cSCC and its precursors is cumulative solar UV exposure (5, 21, 22). Other risk factors include age, fair skin, male sex, and immunosuppression (5, 22, 23). The presence of AK and cSCCIS increases the risk of cSCC (22, 24). Regarding medication, the most convincing evidence is between the usage of thiazide diuretics and elevated cSCC risk (25).

The risk of individual AK or cSCCIS progressing into cSCC is unclear, with estimates varying from 0.025% to 20% for AKs and 3% to 16% for cSCCISs (7, 26–29). At present, there are no means to identify rapidly progressing AKs, even though several clinical features have been postulated to indicate increased risk of progression (30).

AKs and cSCCISs are indicators for increased risk of keratinocyte cancer and considered precursor lesions of cSCC (31). As there are no means to identify AKs that are likely to progress, the design of follow-up is challenging and there is a need for tools to identify those patients with elevated risk of cSCC for closer surveillance among the vast patient population with these premalignant lesions. In this study we investigated clinical risk factors for cSCC development in patient populations with histologically confirmed AK or cSCCIS as their initial lesion.

MATERIALS AND METHODS

Ethical issues

The study was approved by the Ethics Committee of the Hospital District of Southwest Finland (187/2006) and Auria Biobank's Scientific Steering Committee (AB15-9721). The research was carried out according to the Declaration of Helsinki. Registry study approval for collection and use of clinicopathological data was obtained from the Turku University Hospital Clinical Research Centre (TO5/042/18).

Research material

The area served by Turku University Hospital constituted the study region. For cohort creation automated screenings were carried out by Auria Biobank. The first cohort comprised patients with AK as their initial diagnosis without cSCC development during a 5-year observation period, which was compared with a cohort comprising patients with AK as their initial lesion who developed cSCC at any anatomical location during the 5-year observation period. Respectively, a cohort consisting of patients with cSCCIS as their initial lesion without cSCC development during a 5-year observation period was created with a comparison cohort comprising patients with cSCCIS as their initial diagnosis who developed cSCC during the 5-year observation period. In analyses these cohorts were also mixed and patient cohorts with AK and cSCCIS compared regardless of cSCC development, and also patients with either AK or cSCCIS developing cSCC were compared with patients with either AK or cSCCIS not developing cSCC. All AKs, cSCCISs, and cSCCs were histopathologically confirmed by pathologists. As inclusion criteria, patients were not allowed to have either histopathologically confirmed or clinically diagnosed AK, cSCCIS or cSCC before the diagnosis of the inclusive AK or cSCCIS. The histopathological specimens of initial AK or cSCCIS lesions were taken between January 1994 and May 2012. Construction of the cohorts from the automated search to the final cohorts applied in the analyses is presented in **Fig. 1**. Patient records and histopathological reports were manually

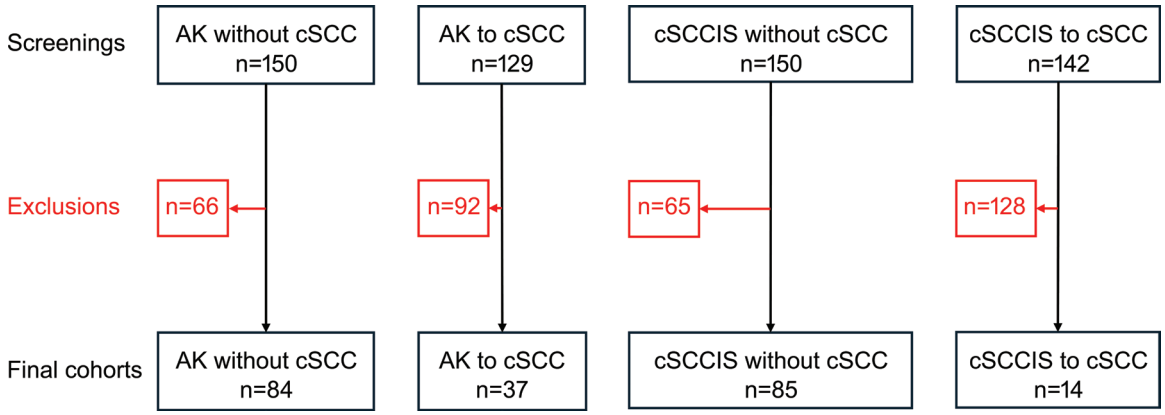

Fig. 1. Flowchart visualizing cohort creation. From automated screenings via manual exclusions to final cohorts. AK: actinic keratosis; cSCC: squamous cell carcinoma; cSCCIS: cutaneous squamous cell carcinoma *in situ*.

reviewed retrospectively from the patient records and pathology database of Turku University Hospital.

Patient and tumour variables

Patient-level characteristics included sex, age at initial AK or cSCCIS diagnosis, comorbidities, medications, occupation, and smoking status. Long term (≥1 year) regular use of medication prior to the histopathological confirmation of initial AK or cSCCIS diagnosis was registered. Farmers, gardeners, construction workers, landscapers, foresters, caretakers, military personnel, fishermen, and seafarers were regarded as high UV-exposure occupations. Solid-organ transplant recipients (SOTRs), patients on immunosuppressive medication, those with HIV infection, chronic lymphocytic leukaemia, or non-Hodgkin's lymphoma were considered immunocompromised.

Collected lesion-level variables included date of the tissue specimen, anatomical location, and the degree of epidermal dysplasia (AK I/II/III).

The 5-year observation period started at the time of the histopathological confirmation of the inclusive AK or cSCCIS.

Statistical analysis

All statistical analysis was performed using IBM SPSS version 28.0 (IBM Corp, Armonk, NY, USA). Bidirectional p-values <0.05 and 95% confidence intervals (CIs) of odds ratios (ORs) not including 1.00 were considered statistically significant. In analyses, every patient was counted once. Baseline patient and tumour characteristics were analysed using descriptive statistics, mainly crosstabs and frequency tabulation. Statistical analyses were conducted with Pearson's χ^2 test and Fisher's exact test. For scale variables, such as age, the Mann–Whitney U test or independent sample t-test was applied. Regarding comorbidities and medications, only variables with n of ≥4 cohorts combined were included in the analyses and are shown in the tables.

Binary logistic regression analyses with 95% CIs were performed in order to determine ORs regarding the risk of cSCC development. Variables were selected for logistic regression analyses if descriptive statistical analyses showed statistical significance or if it was clinically reasonable. Adjusted ORs (aORs) were calculated, including variables with significant crude ORs. Multicollinearity was assessed using a variance inflation factor with values >5 regarded as signs of potential multicollinearity. In cases of multicollinearity the variable for the adjusted model was selected based on clinical reasoning.

Kaplan–Meier cumulative 1 minus survival curve was created to visualize time to cSCC development. Statistical analysis was performed using log-rank (Mantel–Cox) test.

RESULTS

Differences between actinic keratosis and cutaneous squamous cell carcinoma in situ *cohorts*

There were in total 220 patients, of which 121 had AK as their inclusive lesion and 99 cSCCIS as their initial lesion (Fig. 1). Baseline characteristics of the cohorts are indicated in **Table I**. Psoriasis was a more common comorbidity in patients with AK ($p=0.034$) and rheumatoid arthritis in patients with cSCCIS ($p=0.048$). A clear majority (86.0%) of initial AKs were located in the head and neck region in comparison with 56.6% of initial cSCCISs ($p<0.001$). Initial cSCCIS was more frequently located in upper limb, lower limb, or trunk than AK ($p>0.001$) (Table I).

cSCC developed for 37 patients (30.6%) with AK and for 14 patients (14.1%) with cSCCIS as their inclusive lesion. Some 70.3% of cSCCs in the AK cohort developed in the same anatomical region in comparison with 42.9% of cSCCs in the cSCCIS cohort ($p=0.106$) and 29.7% and 21.4% at same exact location respectively ($p=0.730$) (Table I).

Cohort comparisons with regard to cutaneous squamous cell carcinoma development

Fifty-one patients with either AK or cSCCIS as their initial lesion developed cSCC during the 5-year observation period (cSCC cohort) and 169 patients did not (control cohort). A high UV-exposure occupation was more frequent in the cSCC cohort ($p=0.025$). The majority of the patients developing cSCC were males (80.4%) in comparison with a minority in the control cohort (46.2%) ($p<0.001$). A higher proportion of patients who developed cSCC were immunocompromised ($p=0.005$). Coronary artery disease ($p=0.031$) and atrial flutter or fibrillation ($p=0.028$) were more prevalent in patients developing cSCC. Aspirin ($p=0.020$), warfarin ($p=0.003$), and alpha blocker ($p=0.022$) usage were more common in patients who developed cSCC, similar to immunosuppressive medications: ciclosporin ($p<0.001$), azathioprine ($p<0.001$), and prednisolone/prednisone ($p<0.001$). BCC as a comorbidity was more common in the cSCC cohort ($p=0.011$) (**Table II**).

Of 121 patients with AK as their initial lesion, 37 developed cSCC during the 5-year observation period (AK to cSCC cohort) and 84 patients did not (control cohort). Male predominance was observed in AK to cSCC cohort with 31 (83.8%) males in comparison with 36 (42.9%) males in the control cohort ($p<0.001$). Mean age at diagnosis of the initial AK lesion was 71.3 years for patients in the control cohort and 75.6 years in the cSCC cohort ($p=0.031$). There were 6 (7.1%) immunocompromised patients in the control and 8 (21.6%) patients in the AK to cSCC cohort ($p=0.031$). High UV-exposure occupation was more common in the AK to cSCC cohort ($p=0.023$). The majority of initial AKs were located in the head and neck region both in the control (91.7%) and in the AK to cSCC (73.0%) cohorts ($p=0.014$). A higher proportion of initial AK lesions in the AK to cSCC cohort represented a high degree of dysplasia (AK III) (40.5%) in comparison with the control cohort, in which the majority of the lesions exhibited a low degree of dysplasia (AK I) (64.3%) ($p<0.001$). No statistically significant differences were observed in respect of comorbidities between the cohorts. Long-term oral corticosteroid ($p<0.001$), ciclosporin ($p=0.010$), and azathioprine ($p=0.030$) use was more common in the AK to cSCC cohort. The usage of alpha-adrenoreceptor

Table I. Differences between actinic keratosis (AK) and cutaneous squamous cell carcinoma *in situ* (cSCCIS) patients

Characteristics	Total	AK	cSCCIS	*p*-value
Patients in total, *n* (%)	220 (100.0)	121 (100.0)	99 (100.0)	
Age at initial AK diagnosis				
Median, years	74.7	73.3	75.3	
Mean (SD), years	73.5 (10.5)	72.6 (10.1)	74.6 (10.8)	0.157
Range, years	38–91	38–89	40–91	
Occupation				
High UV exposure, *n* (%)	35 (15.9)	17 (14.0)	18 (18.2)	0.446
Low UV exposure, *n* (%)	124 (56.4)	70 (57.9)	54 (54.5)	0.446
Missing, *n* (%)	61 (27.7)	34 (28.1)	27 (27.3)	
Sex				
Male, *n* (%)	119 (54.1)	67 (55.4)	52 (52.5)	0.686
Female, *n* (%)	101 (45.9)	54 (44.6)	47 (47.5)	0.686
Smoking				
Yes, *n* (%)	18 (8.2)	11 (9.1)	7 (7.1)	0.454
No, *n* (%)	138 (62.7)	68 (56.2)	70 (70.7)	0.454
Missing, *n* (%)	64 (29.1)	42 (34.7)	22 (22.2)	
Immunosuppression				
Yes, *n* (%)	29 (13.2)	14 (11.6)	15 (15.2)	0.549
No, *n* (%)	191 (86.8)	107 (88.4)	84 (84.8)	0.549
SOTR, *n* (%)	10 (4.5)	6 (5.0)	4 (4.0)	1.000
HIV, *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)	
Immunosuppressive medication, *n* (%)	26 (11.8)	12 (9.9)	14 (14.1)	0.403
Haematologic malignancy				
CLL, *n* (%)	6 (2.7)	3 (2.5)	3 (3.0)	1.000
Non-Hodgkin lymphoma, *n* (%)	3 (1.4)	2 (1.7)	1 (1.0)	1.000
Comorbidities				
Hypertension, *n* (%)	107 (48.6)	60 (49.6)	47 (47.5)	0.787
MCC, *n* (%)	45 (20.5)	24 (19.8)	21 (21.2)	0.867
Heart failure, *n* (%)	13 (5.9)	8 (6.6)	5 (5.1)	0.776
AF/AFL, *n* (%)	27 (12.3)	15 (12.4)	12 (12.1)	1.000
Earlier stroke, *n* (%)	10 (4.5)	6 (5.0)	4 (4.0)	1.000
Dyslipidaemia, *n* (%)	25 (11.4)	10 (8.3)	15 (15.2)	0.136
Osteoporosis, *n* (%)	8 (3.6)	2 (1.7)	6 (6.1)	0.144
ASO, *n* (%)	4 (1.8)	2 (1.7)	2 (2.0)	1.000
Alzheimer, *n* (%)	7 (3.2)	2 (1.7)	5 (5.1)	0.248
Epilepsy, *n* (%)	4 (1.8)	2 (1.7)	2 (2.0)	1.000
Depression, *n* (%)	6 (2.7)	4 (3.3)	2 (2.0)	0.693
COPD, *n* (%)	7 (3.2)	3 (2.5)	4 (4.0)	0.704
Asthma, *n* (%)	11 (5.0)	6 (5.0)	5 (5.1)	1.000
Rheumatoid arthritis, *n* (%)	7 (3.2)	1 (0.8)	6 (6.1)	0.048
Gout, *n* (%)	10 (4.5)	5 (4.1)	5 (5.1)	0.757
T2DM, *n* (%)	21 (9.5)	12 (9.9)	9 (9.1)	1.000
Hypothyroidism, *n* (%)	17 (7.7)	9 (7.4)	8 (8.1)	1.000
Psoriasis, *n* (%)	6 (2.7)	6 (5.0)	0 (0.0)	0.034
Atopic dermatitis, *n* (%)	7 (3.2)	5 (4.1)	2 (2.0)	0.462
Seborrhoeic dermatitis, *n* (%)	4 (1.8)	4 (3.3)	0 (0.0)	0.129
Medication				
NSAID, *n* (%)	7 (3.2)	2 (1.7)	5 (5.1)	0.248
Paracetamol, *n* (%)	4 (1.8)	2 (1.7)	2 (2.0)	1.000
ASA[a], *n* (%)	61 (27.7)	33 (27.3)	28 (28.3)	0.881
Thiazide diuretic, *n* (%)	22 (10.0)	14 (11.6)	8 (8.1)	0.500
Furosemide, *n* (%)	33 (15.0)	18 (14.9)	15 (15.2)	1.000
ACE/ARB, *n* (%)	59 (26.8)	36 (29.8)	23 (23.2)	0.289
Statin, *n* (%)	56 (25.5)	30 (24.8)	26 (26.3)	0.877
Thyroxine, *n* (%)	16 (7.3)	9 (7.4)	7 (7.1)	1.000
Trimethoprim, *n* (%)	6 (2.7)	5 (4.1)	1 (1.0)	0.227
Neuroleptics, *n* (%)	10 (4.5)	4 (3.3)	6 (6.1)	0.351
SSRI/SNRI, *n* (%)	7 (3.2)	6 (5.0)	1 (1.0)	0.132
Methotrexate, *n* (%)	7 (3.2)	3 (2.5)	4 (4.0)	0.704
Warfarin, *n* (%)	25 (11.4)	16 (13.2)	9 (9.1)	0.397
Dipyridamole, *n* (%)	11 (5.0)	6 (5.0)	5 (5.1)	1.000
Beta blocker, *n* (%)	75 (34.1)	39 (32.2)	36 (36.4)	0.568
Dihydropyridine, *n* (%)	25 (11.4)	14 (11.6)	11 (11.1)	1.000
Amlodipine, *n* (%)	8 (3.6)	3 (2.5)	5 (5.1)	0.472
Allopurinol, *n* (%)	10 (4.5)	6 (5.0)	4 (4.0)	1.000
Metformin, *n* (%)	10 (4.5)	5 (4.1)	5 (5.1)	0.757
5-alphareductase inhibitor, *n* (%)	13 (5.9)	6 (5.0)	7 (7.1)	0.573
Alpha blocker (alfuzosin, tamsulosin), *n* (%)	14 (6.4)	8 (6.6)	6 (6.1)	1.000
Ciclosporin, *n* (%)	9 (4.1)	6 (5.0)	3 (3.0)	0.519
Azathioprine, *n* (%)	8 (3.6)	5 (4.1)	3 (3.0)	0.733
Prednisolone, *n* (%)	21 (9.5)	9 (7.4)	12 (12.1)	0.258
Tretinoin, *n* (%)	9 (4.1)	7 (5.8)	2 (2.0)	0.191
Alendronate, *n* (%)	6 (2.7)	3 (2.5)	3 (3.0)	1.000
Sulphonylurea, *n* (%)	10 (4.5)	5 (4.1)	5 (5.1)	0.757

(*Continued*)

Table I. (Continued) Differences between actinic keratosis (AK) and cutaneous squamous cell carcinoma *in situ* (cSCCIS) patients

Characteristics	Total	AK	cSCCIS	*p*-value
Spironolactone, *n* (%)	7 (3.2)	6 (5.0)	1 (1.0)	0.132
Digoxin, *n* (%)	19 (8.6)	12 (9.9)	7 (7.1)	0.482
Isosorbide mono-/dinitrate, *n* (%)	41 (18.6)	20 (16.5)	21 (21.2)	0.390
Acitretin, *n* (%)	4 (1.8)	4 (3.3)	0 (0.0)	0.129
Memantine, *n* (%)	4 (1.8)	1 (0.8)	3 (3.0)	0.329
PPI, *n* (%)	18 (8.2)	10 (8.3)	8 (8.1)	1.000
Benzodiazepine, *n* (%)	15 (6.8)	12 (9.9)	3 (3.0)	0.059
Inhaled corticosteroid, *n* (%)	8 (3.6)	6 (5.0)	2 (2.0)	0.300
Antihistamine, *n* (%)	4 (1.8)	4 (3.3)	0 (0.0)	0.129
Ipratropium bromide, *n* (%)	5 (2.3)	2 (1.7)	3 (3.0)	0.659
Co-malignancies				
BCC, *n* (%)	74 (33.6)	46 (38.0)	28 (28.3)	0.152
BCC before AK, *n* (%)	40 (18.2)	26 (21.5)	14 (14.1)	0.218
Other than cSCC or BCC, *n* (%)	78 (35.5)	45 (37.2)	33 (33.3)	0.574
Other than cSCC or BCC before AK, *n* (%)	36 (16.4)	22 (18.2)	14 (14.1)	0.467
Lung cancer, *n* (%)	5 (2.3)	3 (2.5)	2 (2.0)	1.000
Urothelial cancer, *n* (%)	5 (2.3)	4 (3.3)	1 (1.0)	0.382
Breast cancer, *n* (%)	17 (7.7)	11 (9.1)	6 (6.1)	0.456
Melanoma, *n* (%)	8 (3.6)	5 (4.1)	3 (3.0)	0.733
Colon cancer, *n* (%)	6 (2.7)	2 (1.7)	4 (4.0)	0.412
Prostate cancer, *n* (%)	20 (9.1)	10 (8.3)	10 (10.1)	0.814
Uterine cancer, *n* (%)	4 (1.8)	2 (1.7)	2 (2.0)	1.000
Initial lesion location				
Head and neck, *n* (%)	160 (72.7)	104 (86.0)	56 (56.6)	<0.001
Trunk, *n* (%)	30 (13.6)	10 (8.3)	20 (20.2)	<0.001
Upper limb, *n* (%)	24 (10.9)	7 (5.8)	17 (17.2)	<0.001
Lower limb, *n* (%)	6 (2.7)	0 (0.0)	6 (6.1)	<0.001
Subsequent cSCC				
Same anatomical region, *n* (%)	32 (62.7)	26 (70.3)	6 (42.9)	0.106
Other anatomical region, *n* (%)	19 (37.3)	11 (29.7)	8 (57.1)	0.106
Same microlocation (<30mm), *n* (%)	14 (27.5)	11 (29.7)	3 (21.4)	0.730

Notably only comorbidities, medications, and co-malignancies with $n \geq 4$ cohorts combined are shown. Same microlocation means that initial lesion and subsequent cutaneous squamous cell carcinoma (cSCC) were located within 30 mm proximity.
[a]Low-dose, daily aspirin (50–250 mg daily) usage for cardiovascular protection was registered on its own and not registered under nonsteroidal anti-inflammatory drugs.
ACE/ARB: angiotensin-converting enzyme inhibitor/angiotensin receptor blockers; AF/AFL: atrial fibrillation/atrial flutter; ASA: aspirin/acetylsalicylic acid; ASO: arteriosclerosis obliterans; BCC: basal cell carcinoma; CLL: chronic lymphocytic leukaemia; COPD: chronic obstructive pulmonary disease; HIV: human immunodeficiency virus; MCC: coronary artery disease; NSAID: nonsteroidal anti-inflammatory drugs; PPI: proton pump inhibitor; SOTR: solid organ transplant recipient; SSRI/SNRI: selective serotonin reuptake inhibitors/serotonin and norepinephrine reuptake inhibitors; SD: standard deviation; T2DM: type 2 diabetes mellitus; UV: ultraviolet.

antagonists was also more common in the AK to cSCC cohort, but when confined to male patients no statistical significance was reached (Table SI).

Of 99 patients with cSCCIS as their initial lesion, 14 patients (14.1%) developed subsequent cSCC (cSCCIS to cSCC cohort) during the 5-year observation period and 85 patients did not (control cohort). In the cSCCIS to cSCC cohort 71.4% of patients were males in comparison with 49.4% in the control cohort ($p=0.156$). There was practically no difference between the cohorts in respect of age at the time of diagnosis of the initial cSCCIS with a mean age of 74.6 years in the control cohort and 74.6 years in the cSCCIS to cSCC cohort ($p=0.960$). A higher proportion of the patients in cSCCIS to cSCC cohort were immunocompromised (35.7%) in comparison with the control cohort (11.8%) ($p=0.036$). Atrial fibrillation or flutter was more prevalent in the cSCCIS to cSCC cohort (35.7%) than in the control cohort (8.2%) ($p=0.012$). Long term usage of ciclosporin ($p=0.002$) and azathioprine ($p=0.002$) was more frequent in the cSCCIS to cSCC cohort. In addition, usage of warfarin ($p=0.003$), beta blockers ($p=0.033$), dihydropyridine ($p=0.047$), metformin ($p=0.019$), and statins ($p=0.046$) was more common in the cSCCIS to cSCC cohort (Table SII).

Factors associated with the risk of cutaneous squamous cell carcinoma development

In order to determine factors associated with the risk of cSCC development, logistic regression analysis was conducted regarding patients with AK or cSCCIS as their initial lesion, and in addition separately for patients with AK and patients with cSCCIS (**Table III**).

Risk factors for cSCC in patients with either AK or cSCCIS as their initial lesion were: immunosuppressive medication (aOR 4.33; 95% CI 1.50–12.52), male sex (aOR 3.69; 95% CI 1.48–9.22), age of at least 82 years (aOR 3.40; 95% CI 1.07–10.79), and BCC as a comorbidity (aOR 2.43; 95% CI 1.14–5.19). Regarding solely patients with AK as inclusive lesion, risk factors for cSCC development comprised: location of initial AK on upper limb (aOR 27.52; 95% CI 2.93–258.84), moderate degree of dysplasia (aOR 13.92; 95% CI 1.21–159.97), age of at least 82 years (aOR 10.26; 95% CI 1.35–77.71), immunosuppressive medication (aOR 11.82; 95% CI 1.69–82.89), and male sex (aOR 6.29; 95% CI 1.56–25.42). Considering solely patients with cSCCIS as their initial lesion, only aspirin as medication was associated with elevated risk of cSCC development (aOR 11.62; 95% CI 1.45–93.29) (Table III).

Table II. Differences between patients with either actinic keratosis (AK) or cutaneous squamous cell carcinoma *in situ* (cSCCIS) who developed cutaneous squamous cell carcinoma (cSCC) and patients who did not

Characteristics	Total	AK or cSCCIS	AK/cSCCIS to cSCC	*p*-value
Patients in total, *n* (%)	220 (100.0)	169 (100.0)	51 (100.0)	
Age at initial AK diagnosis				
Median, years	74.7	74.1	75.2	
Mean (SD), years	73.5 (10.5)	73.0	75.3	0.215
Range, years	38–91	38–90	49–91	
Occupation				
High UV exposure, *n* (%)	35 (15.9)	21 (12.4)	14 (27.5)	0.025
Low or moderate UV exposure, *n* (%)	124 (56.4)	99 (58.6)	25 (49.0)	0.025
Missing, *n* (%)	61 (27.7)	49 (29.0)	12 (23.5)	
Sex				
Male, *n* (%)	119 (54.1)	78 (46.2)	41 (80.4)	<0.001
Female, *n* (%)	101 (45.9)	91 (53.8)	10 (19.6)	<0.001
Smoking				
Yes, *n* (%)	18 (8.2)	16 (9.5)	2 (3.9)	0.739
No, *n* (%)	138 (62.7)	114 (67.5)	24 (47.1)	0.739
Missing, *n* (%)	64 (29.1)	39 (23.1)	25 (49.0)	
Immunosuppression				
Yes, *n* (%)	29 (13.2)	16 (9.5)	13 (25.5)	0.005
No, *n* (%)	191 (86.8)	153 (90.5)	38 (74.5)	0.005
SOTR, *n* (%)	10 (4.5)	2 (1.2)	8 (15.7)	<0.001
HIV, *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
Immunosuppressive medication, *n* (%)	26 (11.8)	14 (8.3)	12 (23.5)	0.006
Haematologic malignancy				
CLL, *n* (%)	6 (2.7)	3 (1.8)	3 (5.9)	0.139
Non-Hodgkin lymphoma, *n* (%)	3 (1.4)	3 (1.8)	0 (0.0)	1.000
Comorbidities				
Hypertension, *n* (%)	107 (48.6)	80 (47.3)	27 (52.9)	0.525
MCC, *n* (%)	45 (20.5)	29 (17.2)	16 (31.4)	0.031
Heart failure, *n* (%)	13 (5.9)	10 (5.9)	3 (5.9)	1.000
AF/AFL, *n* (%)	27 (12.3)	16 (9.5)	11 (21.6)	0.028
Earlier stroke, *n* (%)	10 (4.5)	6 (3.6)	4 (7.8)	0.246
Dyslipidaemia, *n* (%)	25 (11.4)	21 (12.4)	4 (7.8)	0.457
Osteoporosis, *n* (%)	8 (3.6)	7 (4.1)	1 (2.0)	0.685
ASO, *n* (%)	4 (1.8)	2 (1.2)	2 (3.9)	0.230
Alzheimer, *n* (%)	7 (3.2)	5 (3.0)	2 (3.9)	0.664
Epilepsy, *n* (%)	4 (1.8)	2 (1.2)	2 (3.9)	0.230
Depression, *n* (%)	6 (2.7)	5 (3.0)	1 (2.0)	1.000
COPD, *n* (%)	7 (3.2)	7 (4.1)	0 (0.0)	0.357
Asthma, *n* (%)	11 (5.0)	10 (5.9)	1 (2.0)	0.464
Rheumatoid arthritis, *n* (%)	7 (3.2)	6 (3.6)	1 (2.0)	1.000
Gout, *n* (%)	10 (4.5)	8 (4.7)	2 (3.9)	1.000
T2DM, *n* (%)	21 (9.5)	13 (7.7)	8 (15.7)	0.104
Hypothyroidism, *n* (%)	17 (7.7)	14 (8.3)	3 (5.9)	0.768
Psoriasis, *n* (%)	6 (2.7)	5 (3.0)	1 (2.0)	1.000
Atopic dermatitis, *n* (%)	7 (3.2)	6 (3.6)	1 (2.0)	1.000
Seborrhoeic dermatitis, *n* (%)	4 (1.8)	3 (1.8)	1 (2.0)	1.000
Medication				
NSAID, *n* (%)	7 (3.2)	6 (3.6)	1 (2.0)	1.000
Paracetamol, *n* (%)	4 (1.8)	3 (1.8)	1 (2.0)	1.000
ASA, *n* (%)	61 (27.7)	40 (23.7)	21 (41.2)	0.020
Thiazide diuretic, *n* (%)	22 (10.0)	14 (8.3)	8 (15.7)	0.179
Furosemide, *n* (%)	33 (15.0)	24 (14.2)	9 (17.6)	0.655
ACE/ARB, *n* (%)	59 (26.8)	41 (24.3)	18 (35.3)	0.149
Statin, *n* (%)	56 (25.5)	41 (24.3)	15 (29.4)	0.467
Thyroxine, *n* (%)	16 (7.3)	15 (8.9)	1 (2.0)	0.127
Trimethoprim, *n* (%)	6 (2.7)	4 (2.4)	2 (3.9)	0.625
Neuroleptics, *n* (%)	10 (4.5)	6 (3.6)	4 (7.8)	0.246
SSRI/SNRI, n *n* (%)	7 (3.2)	5 (3.0)	2 (3.9)	0.664
Methotrexate, *n* (%)	7 (3.2)	6 (3.6)	1 (2.0)	1.000
Warfarin, *n* (%)	25 (11.4)	13 (7.7)	12 (23.5)	0.003
Dipyridamole, *n* (%)	11 (5.0)	9 (5.3)	2 (3.9)	1.000
Beta blocker, *n* (%)	75 (34.1)	53 (31.4)	22 (43.1)	0.132
Dihydropyridine, *n* (%)	25 (11.4)	16 (9.5)	9 (17.6)	0.130
Amlodipine, *n* (%)	8 (3.6)	5 (3.0)	3 (5.9)	0.391
Allopurinol, *n* (%)	10 (4.5)	8 (4.7)	2 (3.9)	1.000
Metformin, *n* (%)	10 (4.5)	5 (3.0)	5 (9.8)	0.054
5-alphareductase inhibitor, *n* (%)	13 (5.9)	11 (6.5)	2 (3.9)	0.737
Alpha blocker (alfuzosin, tamsulosin), *n* (%)	14 (6.4)	7 (4.1)	7 (13.7)	0.022
In male patients, *n* (%)	14 (11.8)	7 (9.0)	7 (17.1)	0.235
Ciclosporin, *n* (%)	9 (4.1)	1 (0.6)	8 (15.7)	<0.001
Azathioprine, *n* (%)	8 (3.6)	1 (0.6)	7 (13.7)	<0.001
Prednisolone, *n* (%)	21 (9.5)	9 (5.3)	12 (23.5)	<0.001
Tretinoin, *n* (%)	9 (4.1)	5 (3.0)	4 (7.8)	0.217

(*Continued*)

Table II. (Continued) Differences between patients with either actinic keratosis (AK) or cutaneous squamous cell carcinoma *in situ* (cSCCIS) who developed cutaneous squamous cell carcinoma (cSCC) and patients who did not

Characteristics	Total	AK or cSCCIS	AK/cSCCIS to cSCC	*p*-value
Alendronate, *n* (%)	6 (2.7)	4 (2.4)	2 (3.9)	0.625
Sulphonylurea, *n* (%)	10 (4.5)	6 (3.6)	4 (7.8)	0.246
Spironolactone, *n* (%)	7 (3.2)	5 (3.0)	2 (3.9)	0.664
Digoxin, *n* (%)	19 (8.6)	11 (6.5)	8 (15.7)	0.050
Isosorbide mono-/dinitrate, *n* (%)	41 (18.6)	28 (16.6)	13 (25.5)	0.217
Acitretin, *n* (%)	4 (1.8)	3 (1.8)	1 (2.0)	1.000
Memantine, *n* (%)	4 (1.8)	1 (0.6)	3 (5.9)	0.040
PPI, *n* (%)	18 (8.2)	13 (7.7)	5 (9.8)	0.573
Benzodiazepine, *n* (%)	15 (6.8)	10 (5.9)	5 (9.8)	0.347
Inhaled corticosteroid, *n* (%)	8 (3.6)	7 (4.1)	1 (2.0)	0.685
Antihistamine, *n* (%)	4 (1.8)	3 (1.8)	1 (2.0)	1.000
Ipratropiumbromide, *n* (%)	5 (2.3)	5 (3.0)	0 (0.0)	0.592
Co-malignancies				
BCC, *n* (%)	74 (33.6)	49 (29.0)	25 (49.0)	0.011
BCC before AK, *n* (%)	40 (18.2)	28 (16.6)	12 (23.5)	0.300
Other than cSCC or BCC, *n* (%)	78 (35.5)	57 (33.7)	21 (41.2)	0.404
Other than cSCC or BCC before AK, *n* (%)	36 (16.4)	26 (15.4)	10 (19.6)	0.518
Lung cancer, *n* (%)	5 (2.3)	4 (2.4)	1 (2.0)	1.000
Urothelial cancer, *n* (%)	5 (2.3)	3 (1.8)	2 (3.9)	0.329
Breast cancer, *n* (%)	17 (7.7)	16 (9.5)	1 (2.0)	0.130
Melanoma, *n* (%)	8 (3.6)	6 (3.6)	2 (3.9)	1.000
Colon cancer, *n* (%)	6 (2.7)	5 (3.0)	1 (2.0)	1.000
Prostate cancer, *n* (%)	20 (9.1)	12 (7.1)	8 (15.7)	0.091
Uterine cancer, *n* (%)	4 (1.8)	4 (2.4)	0 (0.0)	0.576
Initial lesion location				
Head and neck, *n* (%)	160 (72.7)	125 (74.0)	35 (68.6)	0.375
Trunk, *n* (%)	30 (13.6)	24 (14.2)	6 (11.8)	0.375
Upper limb, *n* (%)	24 (10.9)	15 (8.9)	9 (17.6)	0.375
Lower limb, *n* (%)	6 (2.7)	5 (3.0)	1 (2.0)	0.375

AK or cSCCIS cohort ($n = 169$) includes patients who did not develop cSCC during 5-year observation period and AK/cSCCIS to cSCC cohort ($n = 51$) patients who developed cSCC during the observation period.
ACE/ARB: angiotensin-converting enzyme inhibitor/angiotensin receptor blockers; AF/AFL: atrial fibrillation/atrial flutter; ASA: aspirin/acetylsalicylic acid; ASO: arteriosclerosis obliterans; BCC: basal cell carcinoma; CLL: chronic lymphocytic leukaemia; COPD: chronic obstructive pulmonary disease; HIV: human immunodeficiency virus; MCC: coronary artery disease; NSAID: nonsteroidal anti-inflammatory drugs; PPI: proton pump inhibitor; SOTR: solid organ transplant recipient; SSRI/SNRI: selective serotonin reuptake inhibitors/serotonin and norepinephrine reuptake inhibitors; SD: standard deviation; T2DM: type 2 diabetes mellitus; UV: ultraviolet.

Time to cutaneous squamous cell carcinoma development

Mean time to cSCC development was 752 days when both AK and cSCCIS patients were considered. Within the first year, a higher proportion of patients with cSCCIS as their initial lesion had developed cSCC in comparison with patients with AK as their initial lesion ($p = 0.010$) (**Fig. 2**A, **Table IV**). One-minus Kaplan–Meier curves visualized that the time to cSCC development was remarkably similar regarding patients with severely dysplastic AKs and cSCCISs (Fig. 2B). Among patients developing cSCC within the 5-year observation period, 50% had developed it by 916 days if the initial lesion was AK I, by 901 days if the initial lesion was AK II, by 319 days if the initial lesion was AK III, and by 192 days if the initial lesion was cSCCIS (Fig. 2B, Table IV).

DISCUSSION

AK is the most frequent neoplasia in white populations (7). Both AK and cSCCIS are potential precursors of cSCC and display typical histopathologic and immunohistochemical features of cSCC (7). It has been shown that patients with AK and cSCCIS are at elevated risk of developing cSCC, but the means to identify patients who will develop cSCC are lacking. The aim of this study was to uncover clinical features that would identify patients who will develop cSCC among population of patients with precursor lesions.

First, we analyzed whether patients who develop cSCCIS as their first UV-induced keratinocyte-derived lesion differ from patients who develop AK as their initial lesion. No marked differences were discovered. This notion indicates that from a clinical point of view both AKs and cSCCISs should be considered as *in situ* cSCCs. It is established that AK is cytologically indistinguishable from cSCCIS and harbours numerous molecular alterations common to cSCC (6, 22). However, based on expression profiles there are differences between AKs and cSCCISs that indicate the more malignant nature of cSCCIS in comparison with AK (32). On the molecular level it has been proposed that cytoplasmic HSP70 expression, high expression of activated keratin, and S100 genes could indicate higher invasiveness and act as markers of progression to invasive cSCC (32, 33). Tumor microenvironment (TME) is also critical to cSCC progression (20). Fibroblasts play a pivotal role in the TME and activation of stromal fibroblasts is evident during the progression of cSCC from normal skin to precursors and to invasive cSCC, but there appears to be no difference between AKs and cSCCISs (34). It has been shown that keratinocytes in chronically sun-exposed histologically normal skin harbour driver mutations, especially in *TP53* and *NOTCH1*, that play a key role in early skin carci-

Table III. Factors associated with the risk of cutaneous cell carcinoma (cSCC) development

Factor	Included in logistic regression analysis, positive/total (%)		Risk of cSCC development by variable			
Risk of cSCC of AK or cSCCIS patients	AK or cSCCIS	AK or cSCCIS to cSCC	Unadjusted OR (95% CI)	*p*-value	Adjusted OR (95% CI)	*p*-value
Sex						
Female	91/169 (53.8)	10/51 (19.6)	1 (ref.)		1 (ref.)	
Male	78/169 (46.2)	41/51 (80.4)	4.78 (2.25–10.17)	<0.001	3.69 (1.48–9.22)	0.005
Occupation						
High UV -exposure	21/169 (12.4)	14/51 (27.5)	2.64 (1.18–5.91)	0.018	1.68 (0.63–4.45)	0.299
Low UV exposure	99/169 (58.6)	25/51 (49.0)	1 (ref.)		1 (ref.)	
Missing	49/169 (29.0)	12/51 (23.5)	0.97 (0.45–2.09)	0.938	0.95 (0.38–2.37)	0.905
Age bands, years						
<67	46/169 (27.2)	10/51 (19.6)	1 (ref.)		1 (ref.)	
67–74	43/169 (25.4)	13/51 (25.5)	1.39 (0.55–3.50)	0.484	1.66 (0.54–5.10)	0.374
75–81	42/169 (24.9)	11/51 (21.6)	1.21 (0.47–3.13)	0.702	1.21 (0.36–4.04)	0.752
≥82	38/169 (22.5)	17/51 (33.3)	2.06 (0.84–5.02)	0.113	3.40 (1.07–10.79)	0.038
Comorbidities						
Immunosuppression	16/169 (9.5)	13/51 (25.5)	3.27 (1.45–7.38)	0.004	NA	NA
SOTR	2/169 (1.2)	8/51 (15.7)	15.54 (3.18–75.82)	<0.001	NA	NA
MCC	29/169 (17.2)	16/51 (31.4)	2.21 (1.08–4.51)	0.030	1.45 (0.55–3.86)	0.457
AF/FHL	16/169 (9.5)	11/51 (21.6)	2.63 (1.13–6.11)	0.025	0.58 (0.08–4.44)	0.601
BCC	49/169 (29.0)	25/51 (49.0)	2.36 (1.24–4.47)	0.009	2.43 (1.14–5.19)	0.021
BCC before initial	28/169 (16.6)	12/51 (23.5)	1.55 (0.72–3.33)	0.261	NA	NA
Prostate cancer	12/169 (7.1)	8/51 (15.7)	2.43 (0.94–6.33)	0.068	NA	NA
In males	12/78 (15.4)	8/41 (19.5)	1.33 (0.50–3.58)	0.568	NA	NA
Medication						
ASA	40/169 (23.7)	21/51 (41.2)	2.26 (1.17–4.37)	0.016	2.00 (0.82–4.90)	0.128
Statin	41/169 (24.3)	15/51 (29.4)	1.30 (0.65–2.61)	0.460	NA	NA
Warfarin	13/169 (7.7)	12/51 (23.5)	3.69 (1.56–8.72)	0.003	4.65 (0.51–42.24)	0.172
Beta blocker	53/169 (31.4)	22/51 (43.1)	1.66 (0.87–3.16)	0.122	NA	NA
Dihydropyridine	16/169 (9.5)	9/51 (17.6)	2.05 (0.85–4.97)	0.112	NA	NA
Alpha blocker	7/169 (4.1)	7/51 (13.7)	3.68 (1.23–11.05)	0.020	2.15 (0.60–7.71)	0.239
In males	7/78 (9.0)	7/41 (17.1)	2.09 (0.68–6.43)	0.199	NA	NA
Metformin	5/169 (3.0)	5/51 (9.8)	3.57 (0.99–12.85)	0.052	NA	NA
Immunosuppressive medication	14/169 (8.3)	12/51 (23.5)	3.41 (1.46–7.95)	0.005	4.33 (1.50–12.52)	0.007
Ciclosporin	1/169 (0.6)	8/51 (15.7)	31.26 (3.81–256.69)	0.001	NA	NA
Azathioprine	1/169 (0.6)	7/51 (13.7)	26.73 (3.20–222.98)	0.002	NA	NA
Prednisolone	9/169 (5.3)	12/51 (23.5)	5.47 (2.15–13.90)	<0.001	NA	NA
Methotrexate	6/169 (3.6)	1/51 (2.0)	0.543 (0.06–4.62)	0.576	NA	NA
Digoxin	11/169 (6.5)	8/51 (15.7)	2.67 (1.01–7.06)	0.047	1.98 (0.55–7.11)	0.295
Initial lesion location						
Head and neck	125/169 (74.0)	35/51 (68.6)	1 (ref.)		NA	NA
Trunk	24/169 (14.2)	6/51 (11.8)	0.89 (0.34–2.36)	0.819	NA	NA
Upper limb	15/169 (8.9)	9/51 (17.6)	2.14 (0.87–5.31)	0.100	NA	NA
Lower limb	5/169 (3.0)	1/51 (2.0)	0.71 (0.08–6.32)	0.762	NA	NA
Risk of cSCC of AK patients	AK	AK to cSCC				
Sex						
Female	48/84 (57.1)	6/37 (16.2)	1 (ref.)		1 (ref.)	
Male	36/84 (42.9)	31/37 (83.8)	6.89 (2.60–18.27)	<0.001	6.29 (1.56–25.42)	0.010
Occupation						
High UV exposure	7/84 (8.3)	10/37 (27.0)	3.84 (1.28–11.52)	0.017	1.29 (0.24–6.95)	0.764
Low UV exposure	51/84 (60.7)	19/37 (51.4)	1 (ref.)		1 (ref.)	
Missing	26/84 (31.0)	8/37 (21.6)	0.83 (0.32–2.14)	0.694	1.07 (0.28–4.13)	0.924
Age bands, years						
<67	26/84 (31.0)	5/37 (13.5)	1 (ref.)			
67–74	23/84 (27.4)	11/37 (29.7)	2.49 (0.75–8.23)	0.136	3.97 (0.69–22.67)	0.121
75–81	22/84 (26.2)	9/37 (24.3)	2.13 (0.62–7.29)	0.230	4.57 (0.64–32.41)	0.129
≥82	13/84 (15.5)	12/37 (32.4)	4.80 (1.39–16.55)	0.013	10.26 (1.35–77.71)	0.024
Comorbidities						
Immunosuppression	6/84 (7.1)	8/37 (21.6)	3.59 (1.15–11.23)	0.028	NA	NA
SOTR	1/84 (1.2)	5/37 (13.5)	12.97 (1.46–115.35)	0.022	NA	NA
MCC	14/84 (16.7)	10/37 (27.0)	1.85 (0.73–4.67)	0.192	NA	NA
AF/FHL	9/84 (10.7)	6/37 (16.2)	1.61 (0.53–4.92)	0.401	NA	NA
BCC	28/84 (33.3)	18/37 (48.6)	1.90 (0.86–4.17)	0.112	NA	NA
BCC before initial	16/84 (19.0)	10/37 (27.0)	1.57 (0.64–3.90)	0.327	NA	NA
Prostate cancer	6/84 (7.1)	4/37 (10.8)	1.58 (0.42–5.95)	0.502	NA	NA
In males	6/36 (16.7)	4/31 (12.9)	0.74 (0.19–2.91)	0.667	NA	NA
Medication						
ASA	20/84 (23.8)	13/37 (35.1)	1.73 (0.75–4.02)	0.200	NA	NA
Statin	22/84 (26.2)	8/37 (21.6)	0.78 (0.31–1.95)	0.592	NA	NA
Warfarin	9/84 (10.7)	7/37 (18.9)	1.94 (0.66–5.70)	0.225	NA	NA
Beta blocker	26/84 (31.0)	13/37 (35.1)	1.21 (0.53–2.74)	0.650	NA	NA
Dihydropyridine	9/84 (10.7)	5/37 (13.5)	1.30 (0.41–4.19)	0.658	NA	NA
Alpha blocker	2/84 (2.4)	6/37 (16.2)	7.94 (1.52–41.44)	0.014	5.77 (0.64–51.72)	0.117

(*Continued*)

Table III. (Continued) Factors associated with the risk of cutaneous cell carcinoma (cSCC) development

Factor	Included in logistic regression analysis, positive/total (%)		Risk of cSCC development by variable			
Risk of cSCC of AK or cSCCIS patients	AK or cSCCIS	AK or cSCCIS to cSCC	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	*p*-value
In males	2/36 (5.6)	6/31 (19.4)	4.08 (0.76–21.93)	0.101	NA	NA
Metformin	3/84 (3.6)	2/37 (5.4)	1.54 (0.25–9.64)	0.643	NA	NA
Immunosuppressive medication	4/84 (4.8)	8/37 (21.6)	5.52 (1.54–19.71)	0.009	11.82 (1.69–82.89)	0.013
Ciclosporin	1/84 (1.2)	5/37 (13.5)	12.97 (1.46–115.35)	0.022	NA	NA
Azathioprine	1/84 (1.2)	4/37 (10.8)	10.06 (1.08–93.39)	0.042	NA	NA
Prednisolone	1/84 (1.2)	8/37 (21.6)	22.90 (2.74–191.02)	0.004	NA	NA
Methotrexate	3/84 (3.6)	0/37 (0.0)	NA	NA	NA	NA
Digoxin	6/84 (7.1)	6/37 (16.2)	2.52 (0.75–8.40)	0.134	NA	NA
Initial lesion location						
Head and neck	77/84 (91.7)	27/37 (73.0)	1 (ref.)		1 (ref.)	
Trunk	5/84 (6.0)	5/37 (13.5)	2.85 (0.77–10.62)	0.118	2.04 (0.31–13.22)	0.456
Upper limb	2/84 (2.4)	5/37 (13.5)	7.13 (1.31–38.93)	0.023	27.52 (2.93–258.84)	0.004
Lower limb	0/84 (0.0)	0/37 (0.0)	NA	NA	NA	NA
Degree of dysplasia of AK						
Unknown	16/84 (19.0)	3/37 (8.1)	1 (ref.)		1 (ref.)	
Low (I)	54/84 (64.3)	11/37 (29.7)	1.09 (0.27–4.38)	0.907	0.55 (0.10–3.16)	0.500
Moderate (II)	2/84 (2.4)	8/37 (21.6)	21.3 (2.95–154.55)	0.002	13.92 (1.21–159.97)	0.035
High (III)	12/84 (14.3)	15/37 (40.5)	6.67 (1.57–28.37)	0.010	2.73 (0.46–16.27)	0.271
Risk of cSCC of cSCCIS patients	cSCCIS	cSCCIS to cSCC				
Sex						
Female	43/85 (50.6)	4/14 (28.6)	1 (ref.)		1 (ref.)	
Male	42/85 (49.4)	10/14 (71.4)	2.56 (0.74–8.80)	0.136	0.44 (0.04–4.71)	0.499
Occupation						
High UV exposure	14/85 (16.5)	4/14 (28.6)	2.29 (0.57–9.25)	0.247	NA	NA
Low UV exposure	48/85 (56.5)	6/14 (42.9)	1 (ref.)		NA	NA
Missing	23/85 (27.1)	4/14 (28.6)	1.39 (0.36–5.42)	0.634	NA	NA
Age bands, years						
< 67	20/85 (23.5)	5/14 (35.7)	1.25 (0.32–4.93)	0.750	4.02 (0.24–66.44)	0.331
67–74	20/85 (23.5)	2/14 (14.3)	0.50 (0.09–2.86)	0.435	0.59 (0.02–20.78)	0.769
75–81	20/85 (23.5)	2/14 (14.3)	0.50 (0.09–2.86)	0.435	0.38 (0.02–8.55)	0.540
≥ 82	25/85 (29.4)	5/14 (35.7)	1 (ref.)		1 (ref.)	
Comorbidities						
Immunosuppression	10/85 (11.8)	5/14 (35.7)	4.17 (1.16–14.94)	0.028	4.59 (0.50–42.26)	0.178
SOTR	1/85 (1.2)	3/14 (21.4)	22.91 (2.19–239.93)	0.009	NA	NA
MCC	15/85 (17.6)	6/14 (42.9)	3.50 (1.06–11.58)	0.040	1.59 (0.07–34.18)	0.768
AF/FHL	7/85 (8.2)	5/14 (35.7)	6.19 (1.62–23.62)	0.008	1.16 (0.01–115.30)	0.950
BCC	21/85 (24.7)	7/14 (50.0)	3.05 (0.96–9.70)	0.059	NA	NA
BCC before initial	12/85 (14.1)	2/14 (14.3)	1.01 (0.20–5.11)	0.987	NA	NA
Prostate cancer	6/85 (7.1)	4/14 (28.6)	5.27 (1.27–21.92)	0.022	17.82 (0.77–411.64)	0.072
In males	6/42 (14.3)	4/10 (40.0)	4.00 (0.86–18.51)	0.076	NA	NA
Medication						
ASA	20/85 (23.5)	8/14 (57.1)	4.33 (1.34–13.98)	0.014	11.62 (1.45–93.29)	0.021
Statin	19/85 (22.4)	7/14 (50.0)	3.47 (1.08–11.14)	0.036	1.90 (0.21–17.35)	0.570
Warfarin	4/85 (4.7)	5/14 (35.7)	11.25 (2.55–49.63)	0.001	27.61 (0.17–4626.14)	0.204
Beta blocker	27/85 (31.8)	9/14 (64.3)	3.87 (1.18–12.64)	0.025	2.65 (0.26–26.59)	0.407
Dihydropyridine	7/85 (8.2)	4/85 (28.6)	4.46 (1.11–17.96)	0.036	4.40 (0.37–51.68)	0.239
Alpha blocker	5/85 (5.9)	1/14 (7.1)	1.23 (0.13–11.40)	0.855	NA	NA
In males	5/42 (11.9)	1/10 (10.0)	0.82 (0.09–7.94)	0.866	NA	NA
Metformin	2/85 (2.4)	3/14 (21.4)	11.32 (1.70–75.41)	0.012	15.17 (0.43–531.68)	0.134
Immunosuppressive med	10/85 (11.8)	4/14 (28.6)	3.00 (0.79–11.39)	0.107	NA	NA
Ciclosporin	0/85 (0.0)	3/14 (21.4)	NA	NA	NA	NA
Azathioprine	0/85 (0.0)	3/14 (21.4)	NA	NA	NA	NA
Prednisolone	8/85 (9.4)	4/14 (28.6)	3.85 (0.98–15.14)	0.054	NA	NA
Methotrexate	3/85 (3.5)	1/14 (7.1)	2.10 (0.20–21.77)	0.533	NA	NA
Digoxin	5/85 (5.9)	2/14 (14.3)	2.67 (0.46–15.32)	0.272	NA	NA
Initial lesion location						
Head and neck	48/85 (56.5)	8/14 (57.1)	1 (ref.)		NA	NA
Trunk	19/85 (22.4)	1/14 (7.1)	0.32 (0.04–2.70)	0.292	NA	NA
Upper limb	13/85 (15.3)	4/14 (28.6)	1.85 (0.48–7.10)	0.373	NA	NA
Lower limb	5/85 (5.9)	1/14 (7.1)	1.20 (0.12–11.66)	0.875	NA	NA

Risk of cSCC development regarding different populations; risk of patients with either actinic keratosis (AK) or cSCCIS as their initial lesion, risk of patients with AK as their initial lesion, and risk of patients with cutaneous squamous cell carcinoma *in situ* (cSCCIS) as their initial lesion.
AF/AFL: atrial fibrillation/atrial flutter; ASA: aspirin/acetylsalicylic acid; BCC: basal cell carcinoma; CI: confidence interval; IQR: interquartile range; MCC: coronary artery disease; OR: odds ratio; SOTR: solid organ transplant recipient; UV: ultraviolet.

nogenesis and are present in AKs, cSCCISs, and cSCCs (35,36). It is also evident that mutation of a single gene or pathway does not explain the progression of AK into cSCC, but additional mutations and alterations in the TME are required (20, 35–37). Genomic analyses indicate that both AKs and cSCCISs are true precancerous lesions that harbour driver alterations (36). There are genetic similarities between AK and cSCC; however,

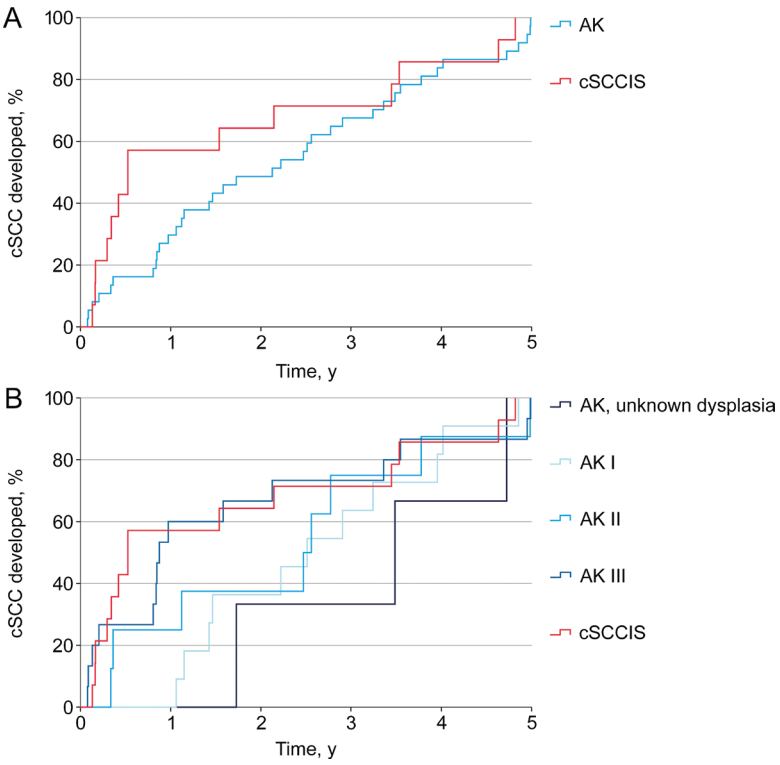

Fig. 2. Time to cutaneous squamous cell carcinoma (cSCC) development from the initial diagnosis of precursor lesion. (A) Actinic keratosis (AK) lesions regardless of grade of dysplasia vs cutaneous squamous cell carcinoma *in situ* (cSCCIS) lesions. (B) AK lesions separated based on dysplasia grade vs cSCCIS lesions. Y-axis: cumulative events (= cSCC development), X-axis: time in years. AK I: actinic keratosis with low-grade dysplasia; AK II: actinic keratosis with moderate-grade dysplasia; AK III: actinic keratosis with high-grade dysplasia.

both AKs and cSCCISs exhibit a low mutational burden in comparison with cSCCs (36, 38).

The main aim of the study was to discover clinicohistopathological features associated with cSCC development within patients harboring either AK or cSCCIS as their initial keratinocyte-derived neoplasia. Classic and well-established patient-related features include advanced age, demonstrating cumulative solar UV-radiation-related damage, male sex, and immunosuppression (1, 22, 39). Our results show that in the population of patients with precursor lesions demonstrating high lifelong UV-radiation exposure, these features distinguish the patients with elevated risk of development of invasive cSCC. More interestingly, we found out that comorbidity with another keratinocyte-derived cutaneous cancer, BCC, also elevated the risk of cSCC development.

Notably, most of the cSCCs are located in the head and neck region (3,39). However, in our study, within the population of patients with AK as their initial lesion it was more probable for the patient to develop cSCC if the initial AK was located on an upper limb.

When solely patients with cSCCIS as their initial lesion were considered, only the usage of aspirin was associated with elevated risk of cSCC development. This is an interesting notion, as it has previously been shown that use of aspirin would, rather, act as chemopreventive agent in the setting of cSCC (40). Furthermore, we have previously noted that the use of aspirin reduces the risk of metastasis from cSCC (3).

Our results also demonstrate that patients with cSCCIS or AK with high-grade dysplasia as their initial lesion develop cSCC faster than patients with AK with a mild or moderate degree of dysplasia.

As for any retrospective study, this study is limited and vulnerable to bias due to data availability. Strict exclusion criteria were applied to the initial cohorts resulting from automated screenings, which led to disproportion in the final cohorts. This study was not

Table IV. Time to cutaneous squamous cell carcinoma (cSCC) development

Time to cSCC development (*n*)	AK + cSCCIS (51)	AK (37)	AK I (10)	AK II (8)	AK III (15)	cSCCIS (14)	*p*-value
Mean (SD), days	752 (598)	813 (578)	954 (471)	837 (595)	617 (624)	591 (641)	0.148
Median, days	577	775	916	917	319	192	
Min, days	29	29	387	123	29	48	
Max, days	1,819	1,819	1,770	1,817	1,819	1,757	
Within first year, *n* (%)	19 (37.3)	11 (29.7)	0 (0.0)	2 (20.0)	9 (60.0)	8 (57.1)	0.010
50% of patients having developed cSCC, SD	577	775	916	901	319	192	

Time to cSCC calculated from the diagnosis of initial precursor (actinic keratosis [AK] or cutaneous squamous cell carcinoma *in situ* [cSCCIS]) lesion. *P*-value regarding mean calculated with Mann–Whitney *U* test between AK and cSCCIS cohorts. *P*-value regarding cSCC development within first year from initial lesion diagnosis calculated with log-rank (Mantel–Cox) test between AK and cSCCIS cohorts.
AK I: actinic keratosis with low grade dysplasia; AK II: actinic keratosis with moderate grade dysplasia; AK III: actinic keratosis with high grade dysplasia; SD: standard deviation.

designed to evaluate the epidemiological risk of cSCC in these patient cohorts.

Our results show that, within the vast population of patients with precursor lesions, male sex, advanced age, immunosuppressive medication, and comorbidity with BCC are associated with elevated risk of cSCC development. We also demonstrate that there are no major differences between the patients who develop cSCCIS as their initial lesion in comparison with patients whose initial lesion is AK. It is also indicated that if the initial lesion is cSCCIS or high-grade dysplastic AK the cSCC will develop more rapidly than in case of AK with a lower degree of dysplasia.

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